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Ambient air — Determination of asbestos fibres — Direct-transfer transmission electron microscopy method

*Air ambiant — Détermination des fibres d'amiante — Méthode de
microscopie électronique à transmission directe*



Reference number
ISO 10312:1995(E)

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Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

International Standard ISO 10312 was prepared by Technical Committee ISO/TC 146, *Air quality*, Subcommittee SC 3, *Ambient atmospheres*.

Annexes A, B, C, D, E and F form an integral part of this International Standard. Annexes G, H and J are for information only.

Introduction

This International Standard is applicable to the determination of airborne asbestos in a wide range of ambient air situations, including the interior atmospheres of buildings, and for detailed evaluation of any atmosphere in which asbestos structures are likely to be present. Because the best available medical evidence indicates that the numerical fibre concentration and the fibre sizes are the relevant parameters for evaluation of the inhalation hazards, a fibre counting technique is the only logical approach. Most fibres in ambient atmospheres are not asbestos, and therefore there is a requirement for fibres to be identified. Many airborne asbestos fibres in ambient atmospheres have diameters below the resolution limit of the optical microscope. This International Standard is based on transmission electron microscopy, which has adequate resolution to allow detection of small fibres and is currently the only technique capable of unequivocal identification of the majority of individual fibres of asbestos. Asbestos is often found, not as single fibres, but as very complex, aggregated structures which may or may not be also aggregated with other particles. The fibres found suspended in an ambient atmosphere can often be identified unequivocally, if a sufficient measurement effort is expended. However, if each fibre were to be identified in this way, the analysis would become prohibitively expensive. Because of instrumental deficiencies or because of the nature of the particulate, some fibres cannot be positively identified as asbestos, even though the measurements all indicate that they could be asbestos. Subjective factors therefore contribute to this measurement, and consequently a very precise definition of the procedure for identification and enumeration of asbestos fibres is required. The method specified in this International Standard is designed to provide the best description possible of the nature, numerical concentration, and sizes of asbestos-containing particles found in an air sample. This International Standard is necessarily complex, because the instrumental techniques used are complex, and also because a very detailed and logical procedure must be specified to reduce the subjective aspects of the measurement. The method of data recording specified in this International Standard is designed to allow re-evaluation of the structure counting data as new medical evidence becomes available. All of the feasible specimen preparation techniques result in some modification of the airborne particulate. Even the collection of particles from a three-dimensional airborne dispersion onto a two-dimensional filter surface can be considered a modification of the particulate, and some of the particles in most samples are modified by the specimen preparation procedures. However, the procedures specified in this International Standard are designed to minimize the disturbance of the collected particulate material, and the effect of those disturbances which do occur can be evaluated.

This International Standard describes the method of analysis for a single air filter. However, one of the largest potential errors in characterizing asbestos in ambient atmospheres is associated with the variability between filter samples. For this reason, it is necessary to design a replicate sampling scheme in order to determine this International Standard's accuracy and precision.

Ambient air — Determination of asbestos fibres — Direct-transfer transmission electron microscopy method

1 Scope

1.1 Substance determined

This International Standard specifies a reference method using transmission electron microscopy for the determination of the concentration of asbestos structures in ambient atmospheres and includes measurement of the lengths, widths and aspect ratios of the asbestos structures. The method allows determination of the type(s) of asbestos fibres present. The method cannot discriminate between individual fibres of the asbestos and non-asbestos analogues of the same amphibole mineral.

1.2 Type of sample

The method is defined for polycarbonate capillary-pore filters or cellulose ester (either mixed esters of cellulose or cellulose nitrate) filters through which a known volume of air has been drawn. The method is suitable for determination of asbestos in both exterior and building atmospheres.

1.3 Measuring range

The range of concentration which can be determined is 50 structures/mm² to 7 000 structures/mm² on the filter. The air concentrations represented by these values are a function of the volume of air sampled. There is no lower limit to the dimensions of asbestos fibres which can be detected. In practice, microscopists vary in their ability to detect very small asbestos fibres. Therefore, a minimum length of 0,5 µm has been defined as the shortest fibre to be incorporated in the reported results.

1.4 Limit of detection

The limit of detection theoretically can be lowered indefinitely by filtration of progressively larger volumes of air and by extending the examination of the specimens in the electron microscope. In practice, the lowest achievable limit of detection for a particular area of TEM specimen examined is controlled by the total suspended particulate concentration.

For total suspended particulate concentrations of approximately 10 µg/m³, corresponding to clean, rural atmospheres, and assuming filtration of 4 000 litres of air, an analytical sensitivity of 0,5 structure/l can be obtained, equivalent to a limit of detection of 1,8 structure/l, if an area of 0,195 mm² of the TEM specimens is examined. If higher total suspended particulate concentrations are present, the volume of air filtered must be reduced in order to maintain an acceptable particulate loading on the filter, leading to a proportionate increase in the analytical sensitivity.

Where this is the case, lower limits of detection can be achieved by increasing the area of the TEM specimens that is examined. In order to achieve lower limits of detection for fibres and bundles longer than 5 µm, and for PCM equivalent fibres, lower magnifications are specified which permit more rapid examination of larger areas of the TEM specimens when the examination is limited to these dimensions of fibre. The direct analytical method cannot be used if the general particulate loading of the sample collection filter exceeds approximately 10 µg/cm² of filter surface, which corresponds to approximately 10 % coverage of the collection filter by particulate. If the total suspended particulate is largely organic material, the limit of detection can be lowered significantly by using an indirect preparation method.

2 Normative references

The following standards contain provisions which, through reference in this text, constitute provisions of this International Standard. At the time of publication, the editions indicated were valid. All standards are subject to revision, and parties to agreements based on this International Standard are encouraged to investigate the possibility of applying the most recent editions of the standards indicated below. Members of IEC and ISO maintain registers of currently valid International Standards.

ISO 4225:1994, *Air quality — General aspects — Vocabulary*

ISO 4226:1993, *Air quality — General aspects — Units of measurement*.

ISO Standard Handbook No. 2:1993, *Quantities and units*.

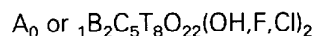
ISO Standard Handbook No. 3:1989, *Statistical Methods*.

3 Definitions

For the purposes of this International Standard, the following definitions apply (see also ISO 4225).

3.1 acicular: The shape of an extremely slender crystal with cross-sectional dimensions which are small relative to its length, i.e. needle-like.

3.2 amphibole: A group of rock-forming ferromagnesium silicate minerals, closely related in crystal form and composition, with the nominal formula:



where

A = K, Na

B = Fe²⁺, Mn, Mg, Ca, Na

C = Al, Cr, Ti, Fe³⁺, Mg, Fe²⁺

T = Si, Al, Cr, Fe³⁺, Ti

In some varieties of amphibole, these elements can be partially substituted by Li, Pb or Zn. Amphibole is characterized by a cross-linked double chain of Si-O tetrahedra with a silicon:oxygen ratio of 4:11, by columnar or fibrous prismatic crystals and by good prismatic cleavage in two directions parallel to the

crystal faces and intersecting at angles of about 56° and 124°.

3.3 amphibole asbestos: Amphibole in an asbestiform habit.

3.4 analytical sensitivity: The calculated airborne asbestos structure concentration in asbestos structures/litre, equivalent to counting of one asbestos structure in the analysis. The method in this International Standard does not specify an analytical sensitivity.

3.5 asbestiform: A specific type of mineral fibrosity in which the fibres and fibrils possess high tensile strength and flexibility.

3.6 asbestos: A term applied to a group of silicate minerals belonging to the serpentine and amphibole groups which have crystallized in the asbestiform habit, causing them to be easily separated into long, thin, strong fibres when crushed or processed. The Chemical Abstracts Service Registry Numbers of the most common asbestos varieties are: chrysotile (12001-29-5), crocidolite (12001-28-4), grunerite asbestos (amosite) (12172-73-5), anthophyllite asbestos (77536-67-5), tremolite asbestos (77536-68-6) and actinolite asbestos (77536-66-4).

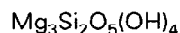
3.7 asbestos structure: A term applied to any connected or overlapping grouping of asbestos fibres or bundles, with or without other particles.

3.8 aspect ratio: The ratio of length to width of a particle.

3.9 blank: A structure count made on TEM specimens prepared from an unused filter, to determine the background measurement.

3.10 camera length: The equivalent projection length between the specimen and its electron diffraction pattern, in the absence of lens action.

3.11 chrysotile: A fibrous mineral of the serpentine group which has the nominal composition



Most natural chrysotile deviates little from this nominal composition. In some varieties of chrysotile, minor substitution of silicon by Al³⁺ may occur. Minor substitution of magnesium by Al³⁺, Fe²⁺, Fe³⁺, Ni²⁺, Mn²⁺ and Co²⁺ may also be present. Chrysotile is the most prevalent type of asbestos.

3.12 cleavage: The breaking of a mineral along one of its crystallographic directions.

3.13 cleavage fragment: A fragment of a crystal that is bounded by cleavage faces.

3.14 cluster: A structure in which two or more fibres, or fibre bundles, are randomly oriented in a connected grouping.

3.15 d-spacing: The distance between identical adjacent and parallel planes of atoms in a crystal.

3.16 electron diffraction: A technique in electron microscopy by which the crystal structure of a specimen is examined.

3.17 electron scattering power: The extent to which a thin layer of substance scatters electrons from their original directions.

3.18 energy dispersive X-ray analysis: Measurement of the energies and intensities of X-rays by use of a solid state detector and multichannel analyser system.

3.19 eucentric: The condition when the area of interest of an object is placed on a tilting axis at the intersection of the electron beam with that axis and is in the plane of focus.

3.20 field blank: A filter cassette which has been taken to the sampling site, opened, and then closed. Such a filter is used to determine the background structure count for the measurement.

3.21 fibril: A single fibre of asbestos, which cannot be further separated longitudinally into smaller components without losing its fibrous properties or appearances.

3.22 fibre: An elongated particle which has parallel or stepped sides. For the purposes of this International Standard, a fibre is defined to have an aspect ratio equal to or greater than 5:1 and a minimum length of 0,5 µm.

3.23 fibre bundle: A structure composed of parallel, smaller diameter fibres attached along their lengths. A fibre bundle may exhibit diverging fibres at one or both ends.

3.24 fibrous structure: A fibre, or connected grouping of fibres, with or without other particles.

3.25 habit: The characteristic crystal growth form, (or combination of these forms), of a mineral, including characteristic irregularities.

3.26 limit of detection: The calculated airborne asbestos structure concentration in structures per li-

tre, equivalent to counting 2,99 asbestos structures in the analysis

3.27 matrix: A structure in which one or more fibres, or fibre bundles, touch, are attached to, or partially concealed by, a single particle or connected group of nonfibrous particles.

3.28 Miller index: A set of either three or four integer numbers used to specify the orientation of a crystallographic plane in relation to the crystal axes.

3.29 PCM equivalent fibre: A fibre of aspect ratio greater than or equal to 3:1, longer than 5 µm, and which has a diameter between 0,2 µm and 3,0 µm.

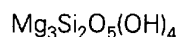
3.30 PCM equivalent structure: A fibrous structure of aspect ratio greater than or equal to 3:1, longer than 5 µm, and which has a diameter between 0,2 µm and 3,0 µm.

3.31 primary structure: A fibrous structure that is a separate entity in the TEM image.

3.32 replication: A procedure in electron microscopy specimen preparation in which a thin copy, or replica, of a surface is made.

3.33 selected area electron diffraction: A technique in electron microscopy in which the crystal structure of a small area of a sample is examined.

3.34 serpentine: A group of common rock-forming minerals having the nominal formula



3.35 structure: A single fibre, fibre bundle, cluster or matrix.

3.36 twinning: The occurrence of crystals of the same species joined together at a particular mutual orientation, such that the relative orientations are related by a definite law.

3.37 unopened fibre: An asbestos fibre bundle of large diameter which has not been separated into its constituent fibrils or fibres.

3.38 zone-axis: The line or crystallographic direction through the centre of a crystal which is parallel to the intersection edges of the crystal faces defining the crystal zone.

4 Principle

A sample of airborne particulate is collected by drawing a measured volume of air through either a

capillary-pore polycarbonate membrane filter of maximum pore size 0,4 µm or a cellulose ester (either mixed esters of cellulose or cellulose nitrate) membrane filter of maximum pore size 0,45 µm by means of a battery-powered or mains-powered pump. TEM specimens are prepared from polycarbonate filters by applying a thin film of carbon to the filter surface by vacuum evaporation. Small areas are cut from the carbon-coated filter, supported on TEM specimen grids, and the filter medium is dissolved away by a solvent extraction procedure. This procedure leaves a thin film of carbon which bridges the openings in the TEM specimen grid, and which supports each particle from the original filter in its original position. Cellulose ester filters are chemically treated to collapse the pore structure of the filter, and the surface of the collapsed filter is then etched in an oxygen plasma to ensure that all particles are exposed. A thin film of carbon is evaporated onto the filter surface and small areas are cut from the filter. These sections are supported on TEM specimen grids and the filter medium is dissolved away by a solvent extraction procedure.

The TEM specimen grids from either preparation method are examined at both low and high magnifications to check that they are suitable for analysis before carrying out a quantitative structure count on randomly-selected grid openings. In the TEM analysis, electron diffraction (ED) is used to examine the crystal structure of a fibre, and its elemental composition is determined by energy dispersive X-ray analysis (EDXA). For a number of reasons, it is not possible to identify each fibre unequivocally, and fibres are classified according to the techniques which have been used to identify them. A simple code is used to record, for each fibre, the manner in which it was classified. The fibre classification procedure is based on successive inspection of the morphology, the electron diffraction pattern for a selected area, and the qualitative and quantitative energy dispersive X-ray analyses. Confirmation of the identification of chrysotile is done only by quantitative ED, and confirmation of amphibole is done only by quantitative EDXA and quantitative zone axis ED.

In addition to isolated fibres, ambient air samples often contain more complex aggregates of fibres, with or without other particles. Some particles are composites of asbestos fibres with other materials. Individual fibres and structures that are more complex are referred to as "asbestos structures". A coding system is used to record the type of fibrous structure, and to provide the optimum description of each of these complex structures. The two codes remove the requirement to interpret the structure counting data from the microscopist, and allow this evaluation to be made later without the requirement for re-

examination of the TEM specimens. Several levels of analysis are specified, the higher levels providing a more rigorous approach to the identification of fibres. The procedure permits a minimum required fibre identification criterion to be defined on the basis of previous knowledge, or lack of it, about the particular sample. Attempts are then made to achieve this minimum criterion for each fibre, and the degree of success is recorded for each fibre. The lengths and widths of all classified structures and fibres are recorded. The number of asbestos structures found on a known area of the microscope sample, together with the equivalent volume of air filtered through this area, is used to calculate the airborne concentration in asbestos structures/litre of air.

5 Symbols of units and abbreviations

5.1 Symbols of units (see also ISO 4226 and ISO No. 2)

eV = electron volt

kV = kilovolt

l/min = litres per minute

µg = microgram (10^{-6} gram)

µm = micrometre (10^{-6} metre)

nm = nanometre (10^{-9} metre)

W = watt

5.2 Abbreviations

DMF	Dimethylformamide
DE	Electron diffraction
EDXA	Energy dispersive X-ray analysis
FWHM	Full width, half maximum
HEPA	High efficiency particle absolute
MEC	Mixed esters of cellulose
PC	Polycarbonate
PCM	Phase contrast optical microscopy
SAED	Selected area electron diffraction
SEM	Scanning electron microscope
STEM	Scanning transmission electron microscope
TEM	Transmission electron microscope

UICC Union Internationale Contre le Cancer

6 Reagents

During the analysis, unless otherwise stated, use only reagents of recognized analytical grade and water (6.1).

WARNING — Use the reagents in accordance with the appropriate health and safety regulations.

6.1 water, fibre-free.

A supply of freshly distilled, fibre-free water, or another source of fibre-free, pyrogen-free water shall be used.

6.2 Chloroform. analytical grade, distilled in glass, preserved with 1 % (V/V) ethanol.

6.3 1-Methyl-2-pyrrolidone.

6.4 Dimethylformamide.

6.5 Glacial acetic acid.

6.6 Acetone.

7 Apparatus

7.1 Air sampling — Equipment and consumable supplies

7.1.1 Filter cassette

Field monitors, comprising 25 mm to 50 mm diameter three-piece cassettes, with cowls which project less than 2 cm in front of the filter surface shall be used for sample collection. The cassette shall be loaded with either a capillary pore polycarbonate filter of maximum pore size 0,4 µm or an MEC or cellulose nitrate filter of maximum pore size 0,45 µm. Either type of filter shall be backed by a 5 µm pore size MEC or cellulose nitrate filter, and supported by a cellulose back-up pad. When the filters are in position, an elastic cellulose band or adhesive tape shall be applied to prevent air leakage. Suitable precautions shall be taken to ensure that the filters are tightly clamped in the assembly, so that significant air leakage around the filter cannot occur.

Representative filters from the filter lot shall be analysed as specified in 9.7 for the presence of asbestos structures before any are used for air sample collection.

7.1.2 Sampling pump

The sampling pump shall be capable of a flow-rate sufficient to achieve the desired analytical sensitivity. The face velocity through the filter shall be between 4,0 cm/s and 25,0 cm/s. The sampling pump used shall provide a non-fluctuating airflow through the filter, and shall maintain the initial volume flow-rate to within ± 10 % throughout the sampling period. A constant flow or critical orifice controlled pump meets these requirements. Flexible tubing shall be used to connect the filter cassette to the sampling pump. A means for calibration of the flow-rate of each pump is also required.

7.1.3 Stand

A stand shall be used to hold the filter cassette at the desired height for sampling, and shall be isolated from the vibrations of the pump (7.1.2).

7.1.4 Variable area flowmeter

A calibrated variable area flowmeter with a range of approximately 1 l/min to 10 l/min is required for calibration of the air sampling system.

The variable area flowmeter shall be cleaned before use to avoid transfer of asbestos contamination from the flowmeter to the sample being collected.

7.2 Specimen preparation laboratory

Asbestos, particularly chrysotile, is present in varying quantities in many laboratory reagents. Many building materials also contain significant amounts of asbestos or other mineral fibres which may interfere with the analysis if they are inadvertently introduced during preparation of specimens. It is most important to ensure that, during preparation, contamination of TEM specimens by any extraneous asbestos fibres is minimized. All specimen preparation steps shall therefore be performed in an environment where contamination of the sample is minimized. The primary requirement of the sample preparation laboratory is that a blank determination shall yield a result which will meet the requirements specified in 9.7. A minimum facility considered suitable for preparation of TEM specimens is a laminar flow hood with positive pressure. However, it has been established that work practices in specimen preparation appear to be more important than the type of clean handling facilities in use. Preparation of samples shall be carried out only after acceptable blank values have been demonstrated.

NOTE 1 It is recommended that activities involving manipulation of bulk asbestos samples not be performed in the

same area as TEM specimen preparation, because of the possibilities of contaminating the TEM specimens.

7.3 Equipment for analysis

7.3.1 Transmission electron microscope

A TEM operating at an accelerating potential of 80 kV to 120 kV, with a resolution better than 1,0 nm, and a magnification range of approximately $\times 300$ to $\times 100\,000$ shall be used. The ability to obtain a direct screen magnification of about $\times 100\,000$ is

necessary for inspection of fibre morphology; this magnification may be obtained by supplementary optical enlargement of the screen image by use of a binocular if it cannot be obtained directly. It is also required that the viewing screen of the microscope be calibrated such that the lengths and widths of fibre images down to 1 mm width can be measured in increments of 1 mm, regardless of image orientation. This requirement is often fulfilled through the use of a fluorescent screen with calibrated gradations in the form of circles, as shown in figure 1.

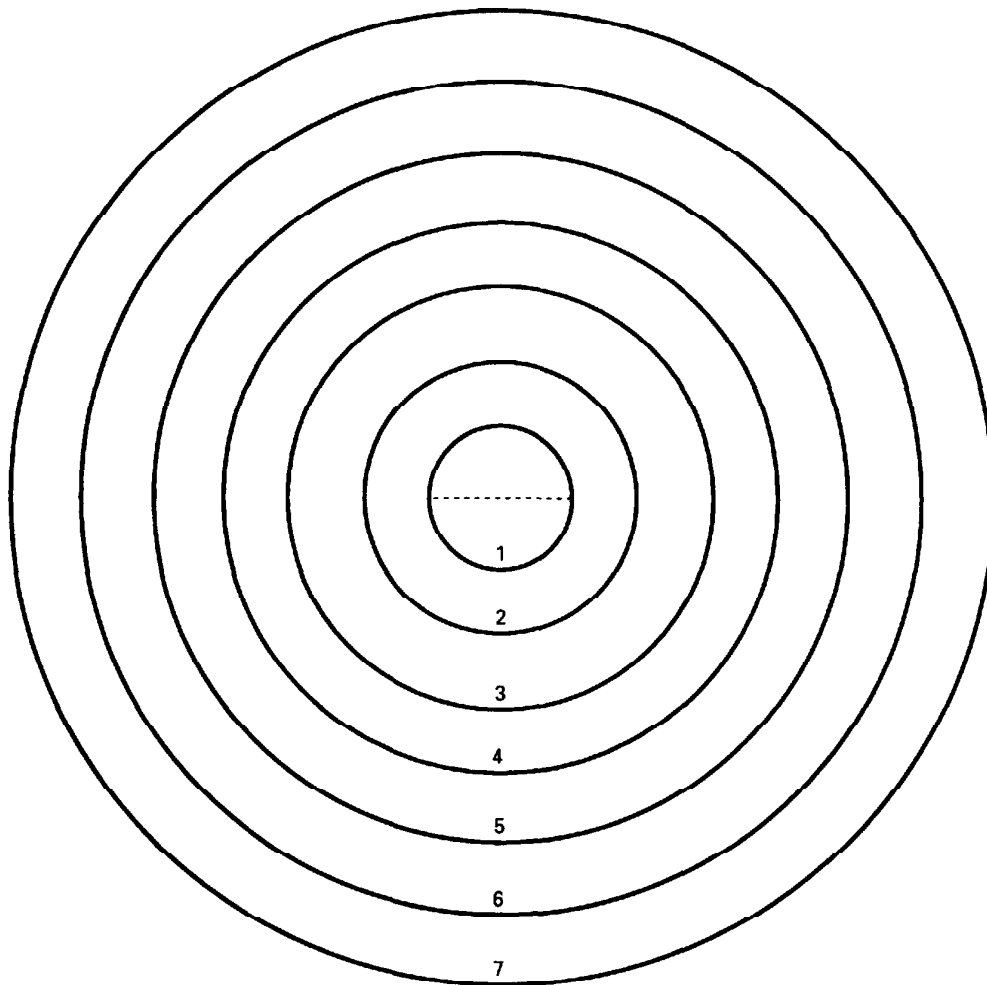


Figure 1 — Example of calibration markings on TEM viewing screen

For Bragg angles less than 0,01 rad, the TEM shall be capable of performing ED from an area of $0,6 \mu\text{m}^2$ or less, selected from an in-focus image at a screen magnification of $\times 20\,000$. This performance requirement defines the minimum separation between particles at which independent ED patterns can be obtained from each particle. If SAED is used, the performance of a particular instrument may normally be calculated using the following equation

$$A = 0,785\,4 \times \left(\frac{D}{M} + 2\,000C_s\theta^3 \right)^2$$

where

- A is the effective SAED area, in square micrometres;
- D is the diameter, in micrometres, of the SAED aperture;
- M is the magnification of the objective lens;
- C_s is the spherical aberration coefficient, in millimetres, of the objective lens;
- θ is the maximum required Bragg angle, in radians.

It is not possible to reduce the effective SAED area indefinitely by the use of progressively smaller SAED apertures, because there is a fundamental limitation imposed by the spherical aberration coefficient of the objective lens.

If zone-axis ED analyses are to be performed, the TEM shall incorporate a goniometer stage which permits the TEM specimen to be either

- rotated through 360° , combined with tilting through at least $+30^\circ$ to -30° about an axis in the plane of the specimen;
- tilted through at least $+30^\circ$ to -30° about two perpendicular axes in the plane of the specimen.

The analysis is greatly facilitated if the goniometer permits eucentric tilting, although this is not essential. If EDXA and zone-axis ED are required on the same fibre, the goniometer shall be of a type which permits tilting of the specimen and acquisition of EDXA spectra without changing the specimen holder.

The TEM shall have an illumination and condenser lens system capable of forming an electron probe of diameter less than 250 nm.

NOTE 2 Use of an anti-contamination trap around the specimen is recommended if the required instrumental performance is to be obtained.

7.3.2 Energy dispersive X-ray analyser

The TEM shall be equipped with an energy dispersive X-ray analyser capable of achieving a resolution better than 180 eV (FWHM) on the $\text{MnK}\alpha$. Since the performance of individual combinations of TEM and EDXA equipment is dependent on a number of geometrical factors, the required performance of the combination of the TEM and X-ray analyser is specified in terms of the measured X-ray intensity obtained from a fibre of small diameter, using a known electron beam diameter. Solid state X-ray detectors are least sensitive in the low energy region, and so measurement of sodium in crocidolite shall be the performance criterion. The combination of electron microscope and X-ray analyser shall yield, under routine analytical conditions, a background-subtracted $\text{NaK}\alpha$ integrated peak count rate of more than 1 count per second (cps) from a fibre of UICC crocidolite, 50 nm in diameter or smaller, when irradiated by an electron probe of 250 nm diameter or smaller at an accelerating potential of 80 kV. The peak/background ratio for this performance test shall exceed 1,0.

The EDXA unit shall provide the means for subtraction of the background, identification of elemental peaks, and calculation of background-subtracted peak areas.

7.3.3 Computer

Many repetitive numerical calculations are necessary, and these may be performed conveniently by relatively simple computer programmes. For analyses of zone-axis ED pattern measurements, a computer with adequate memory is required to accommodate the more complex programmes involved.

7.3.4 Plasma asher

For preparation of TEM specimens from MEC filters, a plasma asher, with a radio frequency power rating of 50 W or higher, shall be used to etch the surface of collapsed MEC filters. The asher shall be supplied with a controlled oxygen flow, and shall be modified, if necessary, to provide a valve to control the speed of air admission so that rapid air admission does not disturb particulates from the surface of the filter after the etching step.

NOTE 3 It is recommended that filters be fitted to the oxygen supply and the air admission line.

7.3.5 Vacuum coating unit

A vacuum coating unit capable of producing a vacuum better than 0,013 Pa shall be used for vacuum deposition of carbon on the membrane filters. A sample

holder is required which will allow a glass microscope slide to be continuously rotated during the coating procedure.

NOTE 4 A mechanism which also allows the rotating slide to be tilted through an angle of approximately 45° during the coating procedure is recommended. A liquid nitrogen cold trap above the diffusion pump may be used to minimize the possibility of contamination of the filter surfaces by oil from the pumping system. The vacuum coating unit may also be used for deposition of the thin film of gold, or other calibration material, when it is required on TEM specimens as an internal calibration of ED patterns.

7.3.6 Sputter coater

A sputter coater with a gold target may be used for deposition of gold onto TEM specimens as an integral calibration of ED patterns. Other calibration materials are acceptable. Experience has shown that a sputter coater allows better control of the thickness of the calibration material.

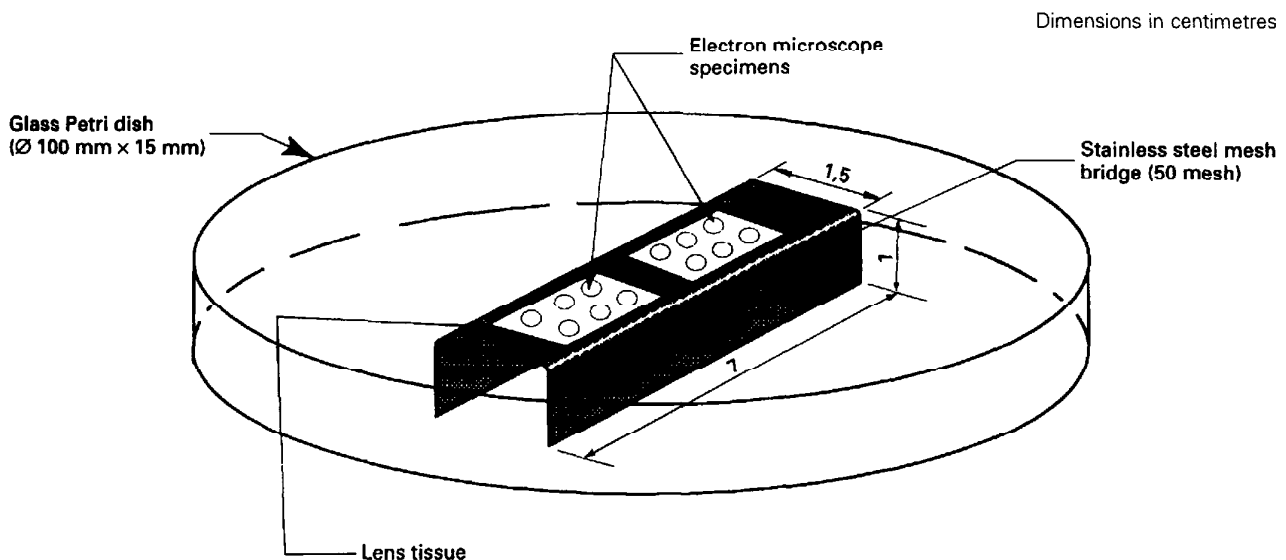
7.3.7 Solvent washer (Jaffe washer)

The purpose of the Jaffe washer is to allow dissolution of the filter polymer while leaving an intact evaporated carbon film supporting the fibres and other particles from the filter surface. One design of

a washer which has been found satisfactory for various solvents and filter media is shown in figure 2. In general, either chloroform or 1-methyl-2-pyrrolidone has been used for dissolving polycarbonate filters and dimethylformamide or acetone has been used for dissolving MEC or cellulose nitrate filters. The higher evaporation rates of chloroform and acetone require that a reservoir of 10 ml to 50 ml of solvent be used, which may need replenishment during the procedure. Dimethylformamide and 1-methyl-2-pyrrolidone have lower vapour pressures and much smaller volumes of solvent may be used. It is recommended that all washers be used in a fume hood, and when specimens are not being inserted or removed, the Petri dish lid shall be in place during the solvent dissolution. The washer shall be cleaned before it is used for each batch of specimens.

7.3.8 Condensation washer

For more rapid dissolution of the filter polymer, or if difficulties are experienced in dissolving the filter polymer, use a condensation washer, consisting of a flask, condenser and cold finger assembly, with a heating mantle and means for controlling the temperature. A suitable assembly is shown in figure 3, using either acetone or chloroform as the solvent, depending on the type of filter.



NOTE — Solvent is added until the meniscus contacts the underside of the stainless steel mesh bridge.

Figure 2 — Example of design of solvent washer (Jaffe washer)

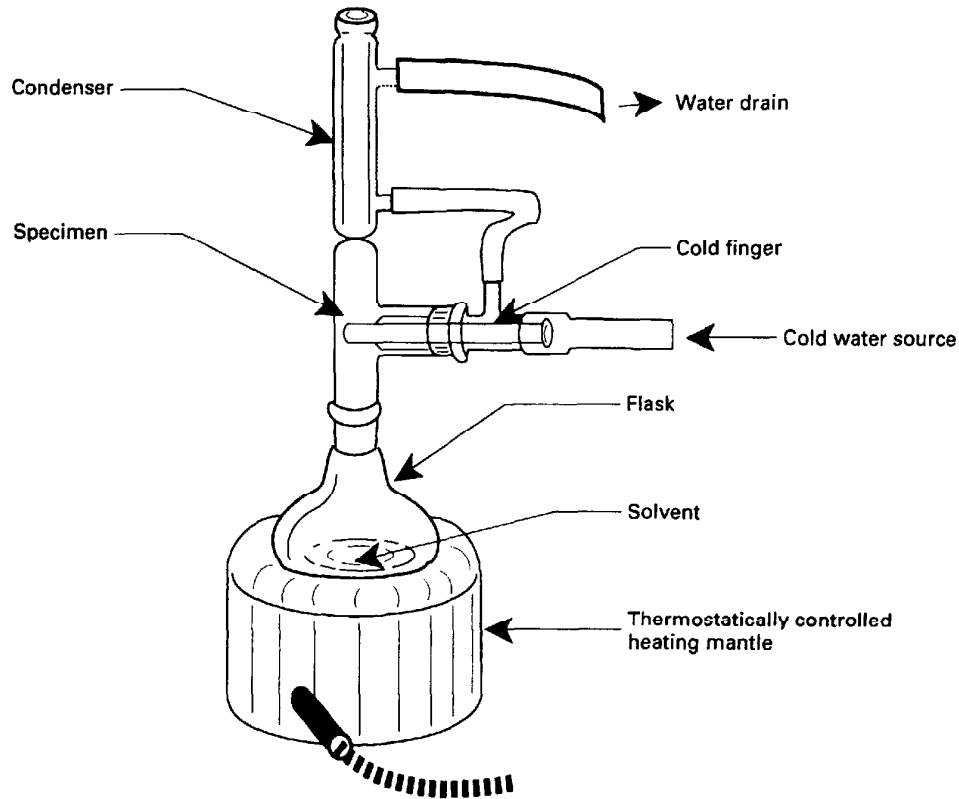


Figure 3 — Example of design of condensation washer

7.3.9 Slide warmer or oven

Use either a slide warmer or an oven for heating slides during the preparation of TEM specimens from MEC or cellulose nitrate filters. It is required to maintain a temperature of 65 °C to 70 °C.

7.3.10 Ultrasonic bath

An ultrasonic bath is necessary for cleaning the apparatus used for TEM specimen preparation.

7.3.11 Carbon grating replica

A carbon grating replica with about 2 000 parallel lines per millimetre shall be used to calibrate the magnification of the TEM.

7.3.12 Calibration specimen grids for EDXA

TEM specimen grids prepared from dispersions of calibration minerals are required for calibration of the EDXA system. Some suitable calibration minerals are riebeckite, chrysotile, halloysite, phlogopite, wollastonite and bustamite. The mineral used for calibration

of the EDXA system for sodium shall be prepared using a gold TEM grid.

7.3.13 Carbon rod sharpener

The use of necked carbon rods, or equivalent, allows the carbon to be evaporated onto the filters with a minimum of heating.

7.3.14 Disposable tip micropipettes

A disposable tip micropipette, capable of transferring a volume of approximately 30 µl, is necessary for the preparation of TEM specimen grids from MEC filters.

7.4 Consumable supplies

7.4.1 Copper electron microscope grids

Copper TEM grids with 200 mesh are recommended. Grids which have grid openings of uniform size such that they meet the requirement specified in 9.6.2 shall be chosen. To facilitate the relocation of individual grid openings for quality assurance purposes, the use of grids with numerical or alphabetical indexing of individual grid openings is recommended.

7.4.2 Gold electron microscope grids

Gold TEM grids with 200 mesh are recommended to mount TEM specimens when sodium measurements are required in the fibre identification procedure. Grids which have grid openings of uniform size such that they meet the requirement specified in 9.6.2 shall be chosen. To facilitate the relocation of individual grid openings for quality assurance purposes, the use of grids with numerical or alphabetical indexing of individual grid openings is recommended.

7.4.3 Carbon rod electrodes

Spectrochemically pure carbon rods, shall be used in the vacuum evaporator (7.3.5) during carbon coating of filters.

7.4.4 Routine electron microscopy tools and supplies

Fine-point tweezers, scalpel holders and blades, microscope slides, double-coated adhesive tape, lens tissue, gold wire, tungsten filaments and other routine supplies are required.

7.4.5 Reference asbestos samples

Asbestos samples, shall be for preparation of reference TEM specimens of the primary asbestos minerals. The UICC set of minerals is suitable for this purpose.

8 Air sample collection

The desired analytical sensitivity is a parameter that shall be established for the analysis prior to sample collection. It is defined as the structure concentration corresponding to the detection of one structure in the analysis. For direct transfer methods of TEM specimen preparation, the analytical sensitivity is a function of the volume of air sampled, the active area of the collection filter, and the area of the TEM specimen over which structures are counted. If total airborne dust levels are high, it may be necessary to terminate sampling before the required volume has been sampled. If this happens, the analytical sensitivity required can be achieved only by counting structures on more grid openings, or by selective concentration of asbestos structures using an indirect TEM specimen preparation technique. Select the sampling rate and the period of sampling to yield the required analytical sensitivity, as detailed in table 1. Before air samples

are collected, unused filters shall be analysed as described in 9.7 to determine the mean asbestos structure count for blank filters.

Air samples shall be collected using filter cassettes (7.1.1). During sampling, the cassette shall be supported on a stand (7.1.3) which is isolated from the vibrations of the pump (7.1.2). The cassette shall be held facing vertically downwards at a height of approximately 1,5 m to 2,0 m above ground/floor level, and shall be connected to the pump with a flexible tube.

Measure the sampling flow-rate at the front end of the cassette, both at the beginning and end of the sampling period, using a calibrated variable area flowmeter (7.1.4) temporarily attached to the inlet of the cassette. The mean value of these two measurements shall be used to calculate the total air volume sampled.

Basic strategies for monitoring environmental sources of airborne asbestos are described in annex G. After sampling, a cap shall be placed over the open end of the cassette, and the cassette packed with the filter face-upwards for return to the laboratory. Field blank filters shall also be included, as specified in 9.7, and submitted to the remaining analytical procedures along with the samples.

NOTES

5 In table 1 a collection filter area of 385 mm² is assumed, and the TEM grid openings are assumed to be 85 µm² square. The limit of detection is defined as the upper 95 % confidence limit of the Poisson distribution for a count of 0 structures. In the absence of background, this is equal to 2,99 times the analytical sensitivity. Backgrounds that are different from 0 observed during analysis of blank filters will degrade the limit of detection.

6 The analytical sensitivity S , expressed in number of structures per litre, is calculated using the following equation:

$$S = \frac{A_t}{kA_gV}$$

where

- A_t is the active area, in square millimetres, of sample collection filter;
- A_g is the mean area, in square millimetres, of grid openings examined;
- k is the number of grid openings examined;
- V is the volume of air sampled, in litres.

Table 1 — Examples of the minimum number of grid openings required to achieve a particular analytical sensitivity and limit of detection

Analytical sensitivity structures/l	Limit of detection structures/l	Volume of air sampled (litres)					
		500	1 000	2 000	3 000	4 000	5 000
0,1	0,30	1 066	533	267	178	134	107
0,2	0,60	533	267	134	89	67	54
0,3	0,90	356	178	89	60	45	36
0,4	1,2	267	134	67	45	34	27
0,5	1,5	214	107	54	36	27	22
0,7	2,1	153	77	39	26	20	16
1,0	3,0	107	54	27	18	14	11
2,0	6,0	54	27	14	9	7	6
3,0	9,0	36	18	9	6	5	4
4,0	12	27	14	7	5	4	4
5,0	15	22	11	6	4	4	4
7,0	21	16	8	4	4	4	4
10	30	11	6	4	4	4	4

9 Procedure for analysis

9.1 General

The techniques used to prepare TEM specimens are different for polycarbonate and cellulose ester filters. The preparation method to be used shall be either 9.3 or 9.4, depending on the type of membrane filter used for air sampling. Cleaning of the sample cassettes before they are opened, preparation of the carbon evaporator, criteria for acceptable specimen grids, and the requirement for blank determinations are identical for the two preparation techniques. TEM examination, structure counting, fibre identification and reporting of results are independent of the type of filter or preparation technique used.

The ability to meet the blank sample criteria is dependent on the cleanliness of equipment and supplies. Consider all supplies such as microscope slides and glassware as potential sources of asbestos contamination. It is necessary to wash all glassware before it is used. Wash any tools or glassware which come into contact with the air sampling filters or TEM specimen preparations both before use and between handling of individual samples. Where possible, disposable supplies should be used.

9.2 Cleaning of sample cassettes

Asbestos fibres can adhere to the exterior surfaces of air sampling cassettes, and these fibres can be inad-

vertently transferred to the sample during handling. To prevent this possibility of contamination, and after ensuring that the cassette is tightly sealed, wipe the exterior surfaces of each sampling cassette before it is placed in the clean facility or laminar flow hood.

9.3 Direct preparation of TEM specimens from polycarbonate filters

9.3.1 Selection of filter area for carbon coating

Use a cleaned microscope slide to support representative portions of polycarbonate filter during the carbon evaporation. Double-coated adhesive tape is used to attach the filter portions to the glass slide. Take care not to stretch the polycarbonate filters during handling. Using freshly cleaned tweezers, remove the polycarbonate filter from the sampling cassette, and place it on to a second cleaned glass microscope slide which is used as a cutting surface. Using a freshly cleaned curved scalpel blade, cut the filter by rocking the blade from the point, pressing it into contact with the filter. Repeat the process as necessary. Several such portions may be mounted on the same microscope slide. The scalpel blade and tweezers shall be washed and dried between the handling of each filter. Identify the filter portions by writing on the glass slide.

9.3.2 Carbon coating of filter portions

Place the glass slide holding the filter portions on the rotation-tilting device, approximately 10 cm to 12 cm

from the evaporation source, and evacuate the evaporator chamber (7.3.5) to a vacuum better than 0,013 Pa. The evaporation of carbon shall be performed in very short bursts, separated by a few seconds to allow the electrodes to cool. If evaporation of carbon is too rapid, the strips of polycarbonate filter will begin to curl, and cross-linking of the surface will occur. This cross-linking procedures a layer of polymer which is relatively insoluble in organic solvents, and it will not be possible to prepare satisfactory TEM specimens. The thickness of carbon required is dependent on the size of particles on the filter, and approximately 30 nm to 50 nm has been found to be satisfactory. If the carbon film is too thin, large particles will break out of the film during the later stages of preparation, and there will be few complete and undamaged grid openings on the specimen. Too thick a carbon film will lead to a TEM image which is lacking in contrast, and the ability to obtain ED patterns will be compromised. The carbon film thickness should be the minimum possible, while retaining most of the grid openings of the TEM specimen intact.

9.3.3 Preparation of the Jaffe washer

Place several pieces of lens tissue, as shown in figure 2, on the stainless steel bridge (7.1.3) and fill the washer (see 7.3.7) with chloroform (6.2) or 1-methyl-2-pyrrolidone (6.3) to a level where the meniscus contacts the underside of the mesh, resulting in saturation of the lens tissue.

9.3.4 Placing of specimens in the Jaffe washer

Using a curved scalpel blade, cut three 3 mm square pieces of carbon-coated polycarbonate filter from the carbon-coated filter portion. Select three squares to represent the centre and the periphery of the active surface of the filter. Place each square of filter, carbon side up, on a TEM specimen grid, and place the grid and filter on the saturated lens tissue in the Jaffe washer. Place the three specimen grids from one sample on the same piece of lens tissue. Any number of separate pieces of lens tissue may be placed in the same Jaffe washer. Cover the Jaffe washer with the lid, and allow the washer to stand for at least 8 h.

NOTE 7 It has been found that some polycarbonate filters will not completely dissolve in the Jaffe washer, even after exposure to chloroform for as long as 3 d. This problem is more severe if the surface of the filter was overheated during the carbon evaporation. It has been found that the problem of residual undissolved filter polymer can be overcome in several ways:

- a) condensation washing of the grids, using chloroform as the solvent, after the initial Jaffe washer treatment, can

often remove much of the residual filter medium in a period of approximately 30 min. To carry out this procedure, transfer the piece of lens tissue supporting the specimen grids to the cold finger of the condensation washer (7.3.8), which has achieved stable operating conditions. Operate the washer for approximately 30 min after inserting the grids;

- b) used in a Jaffe washer, 1-methyl-2-pyrrolidone has been found to be a more effective solvent than chloroform for polycarbonate filters. This solvent is more effective if the lens paper is not used and grids are placed directly on the stainless steel mesh of the Jaffe washer. A dissolution period of 2 h to 6 h has been found to be satisfactory. After dissolution is complete, remove the stainless steel mesh from the Jaffe washer and allow the grids to dry. 1-methyl-2-pyrrolidone evaporates very slowly. If it is required to dry the grids more rapidly, transfer the stainless steel bridge into another Petri dish, and add water (6.1) until the meniscus contacts the underside of the mesh. After approximately 15 min, remove the mesh and allow the grids to dry. If it is desired to retain water-soluble particle species on the TEM grids, ethanol may be used instead of water (6.1) for the second wash;
- c) a mixture of 20 % 1,2-diaminoethane [ethylenediamine] and 80 % 1-methyl-2-pyrrolidone, used in a Jaffe washer, completely dissolves polycarbonate filters in 15 min, even if the surface of the filter has been overheated. To use this solvent, place the grids directly on the stainless steel mesh of the Jaffe washer, do not use the lens paper. After a period of 15 min, transfer the stainless steel bridge into another Petri dish, and add water (6.1) until the meniscus contacts the underside of the mesh. After approximately 15 min, remove the mesh and allow the grids to dry. If it is desired to retain water-soluble particle species on the TEM grids, ethanol may be used instead of water (6.1) for the second wash.

9.3.5 Rapid preparation of TEM specimens from PC filters

TEM specimens can be prepared rapidly from PC filters, if desired, by washing for approximately 1 h in a Jaffe washer, followed by washing for 30 min in a condensation washer using chloroform as the solvent. The alternative filter dissolution procedures described in note 7 may also be used.

9.4 Direct preparation of TEM specimens from cellulose ester filters

9.4.1 Selection of area of filter for preparation

Using clean tweezers, remove the filter from the filter cassette, and place it on a cleaned microscope slide. Using a clean, curved scalpel blade, cut out a portion of the filter.

9.4.2 Preparation of solution for collapsing cellulose ester filters

Mix 35 ml of dimethylformamide (6.4), and 15 ml of glacial acetic acid (6.5) with 50 ml of water (6.1). Store this mixture in a clean bottle. The mixture is stable and suitable for use for up to 3 months after preparation.

9.4.3 Filter collapsing procedure

Using a micropipette with a disposable tip (7.3.14), place 15 $\mu\text{l}/\text{cm}^2$ to 25 $\mu\text{l}/\text{cm}^2$ of the solution prepared in 9.4.2 on a cleaned microscope slide, and using the end of the pipette tip, spread the liquid over the area to be occupied by the filter portion. Place the filter portion, active surface upwards, on top of the solution, lowering the edge of the filter at an angle of about 20° so that air bubbles are not created. Remove any solution not absorbed by the filter by allowing a paper tissue to contact the liquid at the edge of the filter. More than one filter portion may be placed on one slide. Place the slide either on a thermostatically controlled slide warmer (7.3.9) at a temperature of 65 °C to 70 °C, or in an oven (7.3.9) at this temperature, for 10 min. The filter collapses slowly to about 15 % of its original thickness. The procedure leaves a thin, transparent polymer film, with particles and fibres embedded in the upper surface.

9.4.4 Plasma etching of the filter surface

The optimum conditions and time for plasma etching (see 7.3.4) have been determined experimentally from the recovery of fine chrysotile fibrils on 0,8 μm pore size MEC filters. The conditions required in a particular plasma asher shall be established using the procedure specified in annex A. Place the microscope slide holding the collapsed filter portions in the plasma asher, and etch for the time and under the conditions determined. Take care to ensure that the correct conditions are respected. After etching, admit air slowly to the chamber and remove the microscope slide.

Adjust the air admission valve of the plasma asher such that the time taken for the chamber to reach atmospheric pressure exceeds 2 min. Rapid air admission may disturb particulates on the surface of the etched filter.

9.4.5 Carbon coating

Coat the microscope slide holding the collapsed filter portions with carbon as specified in 9.3.2.

9.4.6 Preparation of the Jaffe washer

Place several pieces of lens tissue on the stainless steel bridge, and fill the washer with dimethylformamide (6.4) or acetone (6.6) to a level where the meniscus contacts the underside of the mesh, resulting in saturation of the lens tissue.

9.4.7 Placing of specimens in the Jaffe washer

Place the specimens in the Jaffe washer as specified in 9.3.4. Specimens are normally cleared after approximately 4 h.

9.4.8 Rapid preparation of TEM specimens from cellulose ester filters

An alternative washing procedure may be used to prepare TEM specimens from cellulose ester filters more rapidly than can be achieved by the Jaffe washing procedure. After the specimens have been washed in a Jaffe washer for approximately 1 h, transfer the piece of lens tissue supporting the specimens to the cold finger of a condensation washer (7.3.8) operating with acetone as the solvent because dimethylformamide shall not be used in a condensation washer. Operate the condensation washer for approximately 30 min. This treatment removes all the remaining filter polymer.

9.5 Criteria for acceptable TEM specimen grids

Valid data cannot be obtained unless the TEM specimens meet specified quality criteria. Examine the TEM specimen grid in the electron microscope at a sufficiently low magnification ($\times 300$ to $\times 1\,000$) for complete grid openings to be inspected. Reject the grid if

- a) the TEM specimen has not been cleared of filter medium by the filter dissolution step. If the TEM specimen exhibits areas of undissolved filter medium, and if at least two of the three specimen grids are not cleared, either additional washing with solvent shall be carried out, or new specimens shall be prepared from the filter;
- b) the sample is overloaded with particulate. If the specimen grid exhibits more than approximately 10 % obscuration on the majority of the grid openings, the specimen shall be designated as overloaded. This filter cannot be analysed satisfactorily using the direct preparation methods because the grid is too heavily loaded with debris to allow separate examination of individual particles by ED and EDXA, and obscuration of fibres by

other particulates may lead to underestimation of the asbestos structure count:

- c) the particulate deposits on the specimen are not uniformly distributed from one grid opening to the next. If the particulate deposits on the specimen are obviously not uniform from one grid opening to the next, the specimen shall be designated as non-uniform. This condition is a function either of the air sampling conditions or of the fundamental nature of the airborne particulate. Satisfactory analysis of this filter may not be possible unless a large number of grid openings is examined;
- d) the TEM grid is too heavily loaded with fibrous structures to make an accurate count. Accurate counts cannot be made if the grid has more than approximately 7 000 structures/mm²; or
- e) more than approximately 25 % of the grid openings have broken carbon film over the whole grid opening. Since the breakage of carbon film is usually more frequent in areas of heavy deposit, counting of the intact openings can lead to an underestimate of the asbestos structure count.

NOTE 8 If the specimens are rejected because unacceptable numbers of grid openings exhibit broken carbon replica, an additional carbon coating may be applied to the carbon coated filter, and new specimen grids prepared. The larger particles can often be supported by using a thicker carbon film. If this action does not produce acceptable specimen grids, this filter cannot be analysed using the direct preparation methods.

If one or more of the conditions described in b), c), d) or e) exists, it may not be possible to analyse the sample by this method.

9.6 Procedure for structure counting by TEM

9.6.1 General

The examination consists of a count of asbestos structures which are present on a specified number of grid openings. Fibres are classified into groups on the basis of morphological observations, ED patterns and EDXA spectra. The total number of structures to be counted depends on the statistical precision desired. In the absence of asbestos structures, the area of the TEM specimen grids which must be examined depends on the analytical sensitivity required. The precision of the structure count depends not only on the total number of structures counted, but also on their uniformity from one grid opening to the next. Additional structure counting will be necessary if greater precision is required.

In order that the estimate of the structure density on the sampling filter shall not be based on the small area represented by one specimen grid, grid openings shall be examined on two of the three specimen grids prepared. Then combine the results in the calculation of the structure density. Structure counts shall be made at a magnification of approximately $\times 20\,000$, and shall be terminated at the end of the examination of the grid opening on which the 100th asbestos structure is observed, except that the count shall be continued until a minimum of 4 grid openings have been examined. Otherwise, the structure count shall continue to that number of grid openings at which the specified analytical sensitivity has been achieved.

NOTE 9 The normal range for the number of grid openings which should be examined is from 4 to 20. If insufficient air has been sampled through the filter, the calculation in 9.6.4 can indicate that an impractically large number of grid openings should be examined. When this situation occurs, a larger value of analytical sensitivity may have to be accepted.

9.6.2 Measurement of mean grid opening area

The mean grid opening area shall be measured for the type of TEM specimen grids in use. The standard deviation of the mean of 10 openings selected from 10 grids should be less than 5 %. As an optional procedure, or if the 5 % standard deviation criterion cannot be demonstrated, the dimensions of each grid opening examined in the TEM shall be measured at a calibrated magnification.

9.6.3 TEM alignment and calibration procedures

Before structure counting is performed, align the TEM according to instrumental specifications. Calibrate the TEM and EDXA system according to the procedures specified in annex B.

9.6.4 Determination of stopping point

Before structure counting is begun, calculate the area of specimen to be examined in order to achieve the selected analytical sensitivity. Calculate the maximum number of grid openings to be examined using the following equation:

$$k = \frac{A_f}{A_g VS}$$

where

k is the number of grid openings to be examined, rounded upwards to the next highest integer;

- A_f is the area, in square millimetres, of sample filter;
- A_g is the area, in square millimetres, of TEM specimen grid opening;
- V is the volume of air sampled, in litres;
- S is the required analytical sensitivity, expressed in number of structures per litre.

9.6.5 General procedure for structure counting and size analysis

Use at least two specimen grids prepared from the filter in the structure count. Select at random several grid openings from each grid, and combine the data in the calculation of the results.

Use a form similar to that shown in figure 4 to record the data. Insert the first specimen grid into the TEM.

NOTE 10 In order to facilitate quality assurance measurements which require re-examination of the same grid opening by different microscopists, the grid should be inserted into the specimen holder in a standard orientation with the grid bars parallel and perpendicular to the axis of the specimen holder. This will provide scan directions parallel to the edges of the grid opening. It should be ensured that all microscopists begin scanning at the same starting point on the grid opening, and that they use similar scan patterns. This procedure permits rapid relocation of fibrous structures for further examination if necessary.

Select a typical grid opening and set the screen magnification to the calibrated value (approximately $\times 20\,000$). Adjust the sample height until the features in the centre of the TEM viewing screen are at the eucentric point. Set the goniometer tilt angle to zero. In column 1 of the data recording form, record the number or letter used to identify the grid. In column 2, record the identification of the particular grid opening. Position the specimen so that the grid opening is positioned with one corner visible on the screen. Move the image by adjustment of only one translation control, carefully examining the sample for fibres, until the opposite side of the grid opening is encountered. Move the image by a predetermined distance less than one screen diameter, using the other translation control, and scan the image in the reverse direction. Continue the procedure in this manner until the entire grid opening has been inspected in a pattern similar to that shown in figure 5. When a fibrous structure is detected, assign a sequential number to the primary structure in column 3, perform the identification procedures required as detailed in annex E, and enter the appropriate compositional classification on the structure counting form in column 5. Assign a

morphological classification to the structure according to the procedures specified in annex D, and record this in column 6. Measure on the TEM viewing screen the length and width of the image of the primary structure, in millimetres, and record these measurements in columns 7 and 8. For a disperse cluster or matrix, assign a compositional classification and a morphological classification to each structure component, measure the length and width, and enter the data in columns 4 to 8. Use column 4 of the data recording form to tabulate the sequential number of total structures taking into account structure components. If non-asbestos fibres are observed, note their presence and type, if known. After a fibrous structure has been examined and measured, relocate the original field of view accurately before continuing scanning of the specimen. Failure to do this may cause structures to be overlooked or counted twice. Continue the examination until the completion of the grid opening on which the 100th asbestos structure has been recorded, or until the number of grid openings required to achieve the specified analytical sensitivity, calculated according to 9.6.4, have been examined whichever occurs first. The data shall be drawn approximately equally from a minimum of two grids. Regardless of the value calculated according to 9.6.4, fibrous structures on a minimum of four openings shall be counted.

9.6.6 Measurement of concentration for asbestos fibres and bundles longer than 5 μm

Consider improving the statistical validity for measurement of asbestos fibres and bundles longer than 5 μm by additional examination at a lower magnification, taking account only of the longer fibres and bundles. Perform this extended examination for fibres and bundles longer than 5 μm in accordance with the procedures specified in annex E. Use a magnification of approximately $\times 10\,000$ for counting all asbestos fibres and bundles longer than 5 μm , or approximately $\times 5\,000$ if only fibres and bundles within the diameter range 0,2 μm to 3,0 μm are to be counted. Continue the count until completion of the grid opening on which 100 fibres and bundles have been recorded, or until a sufficient area of the specimen has been examined to achieve the desired analytical sensitivity. Only those structures which are identified as, or are suspected to be, either chrysotile or one of the amphibole minerals will be reported in either the original or the extended TEM examination. Other materials, such as gypsum, cellulose fibres, and filter artifacts such as undissolved filter strands, will not be included in the fibre count. This restriction is intended to ensure that the best statistical validity is obtained for the materials of interest.

TEM asbestos structure count (page of)

Report number: Air volume: litres
 Sample number:
 File name: Sample filter area: mm²
 Sample description:

 Magnification:
 Preparation date: By: Grid opening dimension: µm
 Analysis date: By:
 Computer entry date: By: Level of analysis (C):
 (A):

[illegible]

Figure 4 — Example of structure counting form

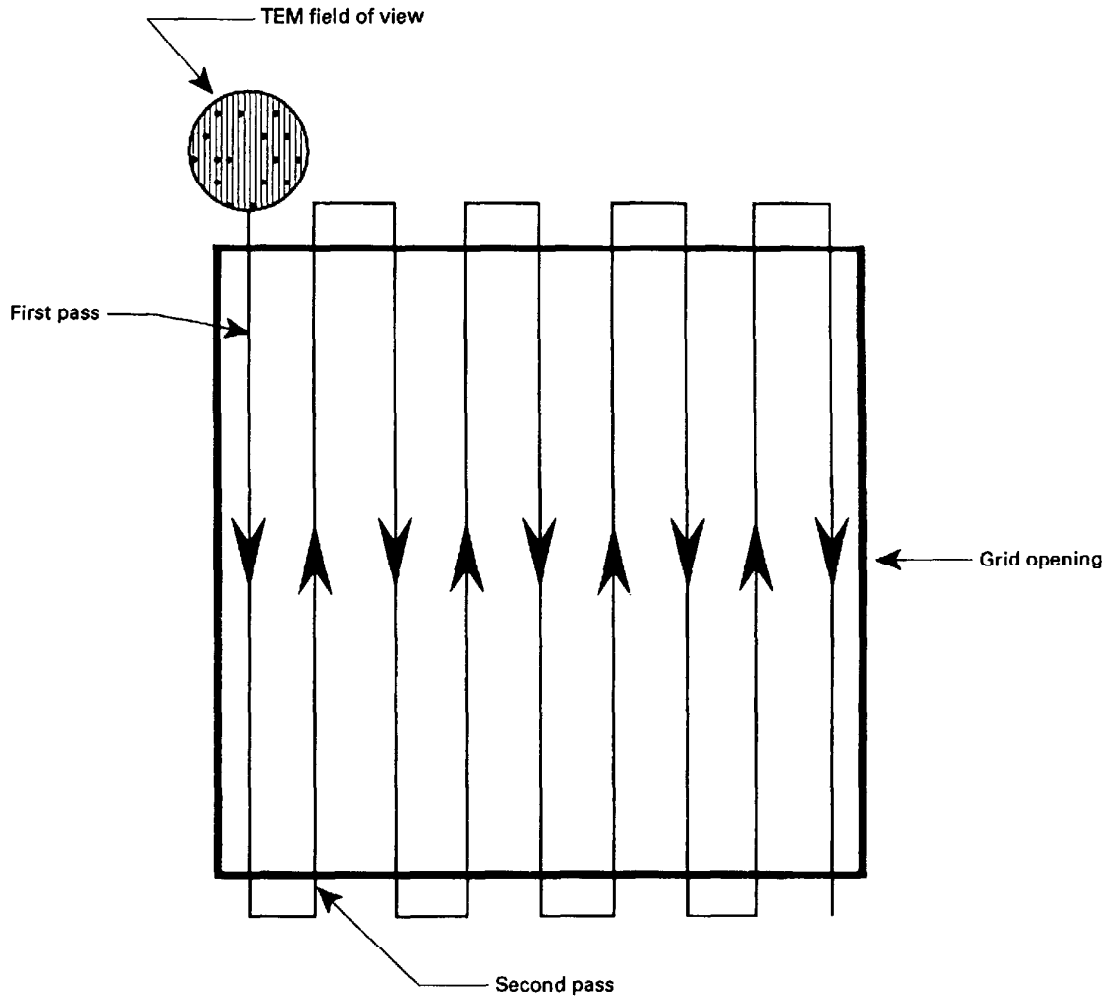


Figure 5 — Example of scanning procedure for TEM specimen examination

9.7 Blank and quality control determinations

Before air samples are collected, a minimum of two unused filters from each filter lot of 100 filters shall be analysed to determine the mean asbestos structure count. If the mean count for all types of asbestos structures is found to be more than 10 structures/mm², or if the mean fibre count for asbestos fibres and bundles longer than 5 µm is more than 0,1 fibre/mm², reject the filter lot.

To ensure that contamination by extraneous asbestos fibres during specimen preparation is insignificant compared with the results reported on samples, establish a continuous programme of blank measurements. At least one field blank shall be processed along with each batch of samples. In addition, at least

one unused filter shall be included with every group of samples prepared on one microscope slide.

Initially, and also at intervals afterwards, ensure that samples of known asbestos concentrations can be analysed satisfactorily. Since there is a subjective component in the structure counting procedure, it is necessary that recounts of some specimens be made by different microscopists, in order to minimize the subjective effects. Such recounts provide a means of maintaining comparability between counts made by different microscopists. Variability between and within microscopists and between laboratories shall be characterized. These quality assurance measurements shall constitute approximately 10 % of the analyses. Repeat results should not differ at the 5 % significance level.

9.8 Calculation of results

Calculate the results using the procedures detailed in annex F. Prior to the TEM examination of the specimens, the level of analysis was specified. Before the results are calculated, the compositional and morphological classifications to be included in the result shall be specified. The chi-squared uniformity test shall be conducted using the number of primary asbestos structures found on each grid opening, prior to the application of the cluster and matrix counting criteria. The concentration result shall be calculated using the numbers of asbestos structures reported after the application of the cluster and matrix counting criteria.

10 Performance characteristics

10.1 General

It is important to use this analytical method in conjunction with a continuous quality control programme. The quality control programme should include use of standard samples, blank samples, and both interlaboratory and intralaboratory analyses.

10.2 Interferences and limitations of fibre identification

Unequivocal identification of every chrysotile fibre is not possible, due to both instrumental limitations and the nature of some of the fibres. The requirement for a calibrated ED pattern eliminates the possibility of an incorrect identification of the fibre selected. However, there is a possibility of misidentification of fibres for which both the morphologies and the ED patterns are reported on the basis of visual inspection only. The only significant possibilities of misidentification occur with halloysite, vermiculite scrolls or palygorskite, all of which can be discriminated from chrysotile by the use of EDXA and by observation of the 0,73 nm (002) reflection of chrysotile in the ED pattern.

As in the case of chrysotile fibres, complete identification of every amphibole fibre is not possible due to instrumental limitations and the nature of some of the fibres. Moreover, complete identification of every amphibole fibre is not practical due to the limitations of both time and cost. Particles of a number of other minerals with compositions similar to those of some amphiboles could be erroneously classified as amphibole when the classification criteria do not include zone-axis ED techniques. However, the requirement for quantitative EDXA measurements on all fibres as support for the random orientation ED technique makes misidentification very unlikely, par-

ticularly when other similar fibres in the same sample have been identified as amphibole by zone-axis methods. The possibility of misidentification is further reduced with increasing aspect ratio, since it is rare for the minerals with which amphibole may be confused to display an asbestiform habit.

10.3 Precision and accuracy (see ISO Standard Handbook No. 3)

10.3.1 Precision

The analytical precision that can be obtained is dependent upon the number of structures counted, and also on the uniformity of the particulate deposit on the original filter. Assuming that the structures are randomly deposited on the filter, if 100 structures are counted and the loading is at least 3,5 structures/grid opening, computer modelling of the counting procedure shows that a coefficient of variation of about 10 % can be expected. As the number of structures counted decreases, the precision will also decrease approximately as \sqrt{N} , where N is the number of structures counted. In practice, particulate deposits obtained by filtration of ambient air samples are rarely ideally distributed, and it is found that the precision is correspondingly reduced. Degradation of precision is a consequence of several factors, such as:

- a) non-uniformity of the filtered particulate deposit;
- b) distortion of the fibre distribution by application of the structure counting criteria;
- c) variation between microscopists in their interpretation of the fibrous structures;
- d) variation between microscopists in their ability to detect and identify fibres.

The 95 % confidence interval about the mean for a single structure concentration measurement using this analytical method should be approximately ± 25 % when 100 structures are counted over 10 grid openings.

10.3.2 Accuracy

There is no independent method available to determine the accuracy.

NOTE 11 It has been demonstrated that, after polycarbonate membrane filters have been coated with carbon, particulate material is transferred to the TEM specimens without measurable losses. However, if the filters are heavily loaded by particulate material, some of this may be lost before they are coated with carbon. Good comparability between the capillary-pore polycarbonate pro-

cedure and the cellulose ester filter procedure has been demonstrated for laboratory-generated aerosols of chrysotile asbestos.

10.3.3 Interlaboratory and intralaboratory analyses

Interlaboratory and intralaboratory analyses are required in order to monitor systematic errors that may develop among microscopists when using this method. These analyses should be designed to test both the overall method and the performance of individual microscopists. Repeating preparation of TEM grids from different sectors of a filter, followed by examination of the grids by a different microscopist, is a test for the reproducibility of the whole method. However, non-uniformity of the particulate deposit on the filter may lead to differences which are not related to the performance of the microscopists. Verified fibre counting (counting of asbestos structures on the same grid opening of a TEM grid by two or more operators, followed by resolution of any discrepancies) may be used both as a training aid and to determine the performance of different microscopists. The use of indexed TEM grids as described in 7.4.1 and 7.4.2 is recommended in order to facilitate relocation of specific grid openings.

10.4 Limit of detection

The limit of detection of the method can be varied by choice of the area of the collection filter, the volume of air sampled and the area of the specimen examined in the TEM. It is also a function of the background of asbestos structures on unused filters. A limit of detection shall be quoted for each sample analysis.

NOTE 12 In practice, the lowest limit of detection is frequently determined by the total suspended particulate concentration, since each particle on the filter must be separated from adjacent ones by a distance large enough for the particle to be identified without interference. Particulate loadings on sampling filters greater than $25 \mu\text{g}/\text{cm}^2$ usually preclude preparation of TEM specimens by the direct methods. If the analysis is to be performed with an acceptable expenditure of time, the area of the specimen examined in the TEM for structures of all sizes is limited in most cases to between 10 and 20 grid openings. In typical ambient or building atmospheres, it has been found that an analytical sensitivity of 1 structure/l can be achieved. In some circumstances, where the atmosphere is exceptionally clean, this can be reduced to 0,1 structure/l or lower. For fibres and bundles longer than $5 \mu\text{m}$, the reduced magnifications specified permit larger areas of the TEM specimens to be examined with an acceptable expenditure of time, resulting in proportionately lower limits of detection. If no structures are found in the analysis, the upper 95 % confidence limit can be quoted as the upper

boundary of the concentration, corresponding to 2,99 times the analytical sensitivity if a Poisson distribution of structures on the filter is assumed. This 95 % confidence limit for 0 structures counted is taken as the detection limit. Since there is sometimes contamination of unused samples filters by asbestos structures, this should also be taken into account in the discussion of limits of detection.

11 Test report

The test report shall include at least the following information:

- a) reference to this International Standard;
- b) identification of the sample;
- c) the date and time of sampling, and all necessary sampling data;
- d) the date of the analysis;
- e) the identity of the analyst;
- f) any procedure used that is not specified in this International Standard or regarded as optional;
- g) a complete listing of the structure counting data (the following data should be included: grid opening number, structure number, identification category, structure type, length and width of the structure in micrometres, and any comments concerning the structure);
- h) a statement of the minimum acceptable identification category and the maximum identification category attempted (refer to tables D.1 and D.2);
- i) a statement specifying which identification and structure categories have been used to calculate the concentration values;
- j) separate concentration values for chrysotile and amphibole structures, expressed in number of asbestos structures per litre;
- k) the 95 % confidence interval limits for the concentration values, expressed in number of asbestos structures per litre;
- l) the analytical sensitivity, expressed in number of asbestos structures per litre;
- m) the limit of detection, expressed in number of asbestos structures per litre;
- n) compositional data for the principal varieties of amphibole, if present;

o) items g) to m) for asbestos fibres and bundles longer than 5 µm;

p) items g) to m) for PCM equivalent asbestos fibres and bundles.

An example of a suitable format for the structure counting data is shown in figures 6 and 7.

Sample analysis information (page 1)

Laboratory name	Report number	Date
Sample: 456 Queen Street Ashby de la Zouch Exterior sample 1991-09-09		
Air volume:		2 150,0 litres
Area of collection filter:		385,0 mm ²
Level of analysis (chrysotile):		CD or CMQ
Level of analysis (amphibole):		ADQ
Magnification used for fibre counting:		× 20 500
Aspect ratio for fibre definition:		5/1
Mean dimension of grid openings:		95,4 µm
Initials of analyst:		JMW
Number of grid openings examined:		10
Analytical sensitivity:		1,968 structures/l
Number of primary asbestos structures:		13
Number of asbestos structures counted:		26
Number of asbestos structures > 5 µm :		7
Number of asbestos fibres and bundles > 5 µm :		10
Number of PCM equivalent asbestos structures:		3
Number of PCM equivalent asbestos fibres:		5

Figure 6 — Example of format for reporting sample and preparation data

Sample analysis information (pages 2 and following)

Laboratory name

Report number

Date

Sample: 456 Queen Street
Ashby de la Zouch
Exterior sample 1991-09-09

TEM asbestos structure count — Raw data

Grid	Grid opening	Number of structures		Identification ¹⁾	Structure type	Length µm	Width µm	Comments
		primary	total					
A	F4-4	1	1	CD	F	1,7	0,045	Crocidolite
		2	2	CMQ	B	2,6	0,09	
		3	3	ADQ	F	4,0	0,15	
	E3-6	4	4	CD	MC+0	3,5	1,3	
	E5-1	5		CD	MD43	7,5	5,0	
			5	CD	MB	7,7	0,30	
			6	CMQ	MF	5,6	0,045	
			7	CD	MB	5,1	0,30	
B	F4-1	6	8	CD	MF	1,7	0,045	
				CD	CD+0	6,5	3,0	
			9	CD	CB	3,5	0,15	
			10	CD	CF	3,5	0,045	
			11	CMQ	CR+0	2,6	1,9	
	G5-1	7		CD	CD31	6,1	3,2	
			12	CD	CB	5,6	0,3	
			13	CMQ	CF	4,0	0,045	
C	E4-4	8	15	CD	B	1,5	0,23	Tremolite
		9	16	AD	F	8,7	0,15	
	G4-4	10		CMQ	CD42	25	5,6	
			17	CMQ	CB	15	0,15	
			18	CMQ	CF	9,4	0,045	
			19	ADQ	CF	3,6	0,30	
			20	CM	CF	4,2	0,045	
	E4-4 E5-6	11		No fibres				
				ADQ	CD+3	9,4	2,5	
			21	ADQ	CF	7,1	0,30	
			22	ADQ	CF	6,2	0,10	
			23	CM	CB	5,1	0,2	
	F4-1	12	24	CM	CR+0	3,3	1,8	
			25	CMQ	MC10	3,7	2,1	
			26	CD	CC+0	7,4	0,5	

1) Identification codes listed in tables D.1 and D.2.

Figure 7 — Example of format for reporting structure counting data

Annex A

(normative)

Determination of operating conditions for plasma asher

A.1 General

During the preparation of TEM specimens from an MEC or cellulose nitrate filter, the spongy structure of the filter is collapsed into a thinner film of polymer by the action of a solvent. Some of the particles on the surface of the original filter become completely buried in the polymer, and the specimen preparation procedure incorporates a plasma etching step to oxidize the surface layer of the polymer. Particles buried by the filter collapsing step are then exposed so that they can become subsequently affixed to the evaporated carbon film without altering their position on the original filter. The amount of etching is critical, and individual ashers vary in performance. Therefore, the plasma asher (7.3.4) shall be calibrated to give a known amount of etching of the surface of the collapsed filter. This is carried out by adjusting the radio-frequency power output and the oxygen flow-rate, and measuring the time taken to completely oxidize an uncollapsed cellulose ester filter with 25 mm diameter of the same type and pore size as those used in the analysis.

A.2 Procedure

Place an unused cellulose ester filter, with 25 mm diameter, of the same type as that being used, in the centre of a glass microscope slide. Position the slide approximately in the centre of the asher chamber. Close the chamber and evacuate to a pressure of approximately 40 Pa, while admitting oxygen to the chamber at a rate of 8 ml/min to 20 ml/min. Adjust the tuning of the system so that the intensity of the plasma is maximized. Measure the time required for complete oxidation of the filter. Determine operating parameters which result in complete oxidation of the filter in a period of approximately 15 min. For etching of collapsed filters, these operating parameters shall be used for a period of 8 min.

NOTE 13 Plasma oxidation at high radio-frequency powers will cause the filter to shrink and curl, followed by sudden violent ignition. At lower powers, the filter will remain in position and will slowly become thinner until it is nearly transparent. It is recommended that a radio-frequency power be used such that violent ignition does not occur. When multiple filters are etched, the rate of etching is reduced, and the system should be calibrated accordingly.

Annex B (normative)

Calibration procedures

B.1 Calibration of the TEM

B.1.1 Calibration of TEM screen magnification

The electron microscope should be aligned according to the specifications of the manufacturer. Initially, and at regular intervals, calibrate the magnifications used for the analysis using a diffraction grating replica (7.3.11). Adjust the specimen height to the eucentric position before carrying out the calibration. Measure the distance on the fluorescent viewing screen occupied by a convenient number of repeat distances of the grating image, and calculate the magnification. Always repeat the calibration after any instrumental maintenance or change of operating conditions. The magnification of the image on the viewing screen is not the same as that obtained on photographic plates or film. The ratio between these is a constant value for the particular model of TEM.

B.1.2 Calibration of ED camera constant

Calibrate the camera constant of the TEM when used in ED mode. Use a specimen grid supporting a carbon film on which a thin film of gold has been evaporated or sputtered. Form an image of the gold film with the specimen adjusted to the eucentric position and select ED conditions. Adjust the objective lens current to optimize the pattern obtained, and measure the diameters of the innermost two rings either on the fluorescent viewing screen or on a recorded image. Calculate the radius-based camera constant, λL , for both the fluorescent screen and the photographic plate or film, using the following equation:

$$\lambda L = \frac{aD}{2,0\sqrt{h^2 + k^2 + l^2}}$$

where

- λ is the wavelength, in nanometres, of the incident electrons;
- L is the camera length, in millimetres;

a is the unit cell dimension of gold, in nanometres (= 0,407 86 nm);

D is the diameter, in millimetres, of the (hkl) diffraction ring.

Using gold as the calibration material, the radius-based camera constant is given by

$$\lambda L = 0,117\ 74D \text{ mm}\cdot\text{nm (smallest ring)}$$

$$\lambda L = 0,101\ 97D \text{ mm}\cdot\text{nm (second ring)}$$

B.2 Calibration of the EDXA system

Energy calibration of the EDXA system for a low energy and high energy peak shall be performed regularly. Calibration of the intensity scale of the EDXA system permits quantitative composition data, at an accuracy of about 10 % of the elemental concentration, to be obtained from EDXA spectra of reference silicate minerals involving the elements Na, Mg, Al, Si, K, Ca, Mn and Fe, and applicable certified reference materials. If quantitative determinations are required for minerals containing other elements, reference standards other than those referred to below will be required. Well-characterized mineral standards permit calibration of any TEM-EDXA combination which meets the instrumental specifications of 7.3.1 and 7.3.2, so that EDXA data from different instruments can be compared. Reference minerals are required for the calibration; the criteria for selection being that they should be silicate minerals with matrices as close as possible to those of the amphiboles or serpentine, and that small individual fragments of the minerals are homogeneous in composition within a few percent.

Determine the compositions of these standards by electron microprobe analysis or chemicals methods. Crush fragments of the same selected mineral standards and prepare filters by dispersal of the crushed material in water and immediate filtration of the suspensions. Prepare TEM specimens from these filters according to the procedures specified in clause 9. These TEM specimens can then be used to calibrate any TEM-EDXA system so that comparable composi-

tional results can be obtained from different instruments.

NOTES

14 The microprobe analysis of the mineral standards are carried out by conventional techniques which can be found in annex J. The mineral is first embedded in a mount of poly(methyl methacrylate) or epoxy resin. The mount is then ground and polished to achieve a flat, polished surface of the mineral fragment. This surface is then analysed, using suitable reference standards, preferably oxide standards of the individual elements wherever these are available. It is necessary to take into account the water concentration in the minerals, which in the case of chrysotile amounts to 13 % by mass. This water content may vary due to losses in the vacuum system.

15 Aqueous suspensions of mineral standards should be filtered immediately after preparation, since alkali and alkali earth metals may be partially leached from minerals containing these elements.

Express the results of the electron microprobe analyses as atomic or mass percentage ratios relative to silicon. X-ray peak ratios of the same elements relative to silicon, obtained from the EDXA system, can then be used to calculate the relationship between peak area ratio and atomic or mass percentage ratio. The technique was described by Cliff and Lorimer (see annex J, reference [8]).

The X-rays generated in a thin specimen by an incident electron beam have a low probability of interacting with the specimen. Thus, mass absorption and fluorescence effects are negligible. In a silicate mineral specimen containing element i , the following equation can be used to perform quantitative analyses in the TEM:

$$\frac{C_i}{C_{Si}} = k_i \times \frac{A_i}{A_{Si}}$$

where

- C_i is the concentration or atomic percentage of element i ;
- C_{Si} is the concentration or atomic percentage of silicon;

A_i is the elemental integrated peak area for element i ;

A_{Si} is the elemental integrated peak area for silicon;

k_i is the k -ratio for element i relative to silicon.

For a particular instrumental configuration and a particular particle size, the value of k_i is constant.

To incorporate correction for the particle size effect on peak area ratios (see annex J, references [35] and [36]), extend the Cliff and Lorimer technique by obtaining separate values of the constant k_i for different ranges of fibre diameter. It is recommended that 20 EDXA measurements be made for each range of fibre diameters. Suitable ranges of fibre diameter are:

< 0,25 μm ; 0,25 μm to 0,5 μm ; 0,5 μm to 1,0 μm ;
 > 1,0 μm .

Insert the TEM grid into the transmission electron microscope, obtain an image at the calibrated higher magnification of about $\times 20\,000$, and adjust the specimen height to the eucentric point. If the X-ray detector is a side-entry variety, tilt the specimen towards the X-ray detector. Select an isolated fibre or particle less than 0,5 μm in width, and accumulate an EDXA spectrum using an electron probe of suitable diameter. When a well-defined spectrum has been obtained, perform a background subtraction and calculate the background-corrected peak areas for each element listed, using energy windows centred on the peaks. Calculate the ratio of the peak area for each specified element relative to the peak area for silicon. All background-subtracted peak areas used for calibration shall exceed 400 counts.

Repeat this procedure for 20 particles of each mineral standard. Reject analyses of any obviously foreign particles. Calculate the arithmetic mean concentration to peak area ratio, k_i (k -ratio), for each specified element of each mineral standard and for each of the fibre diameter ranges. Periodic routine checks shall be carried out to ensure that there has been no degradation of the detector performance. These k -ratios are used to calculate the elemental concentrations of unknown fibres, using the Cliff and Lorimer relationship.

Annex C (normative)

Structure counting criteria

C.1 General

In addition to isolated fibres, other assemblages of particles and fibres frequently occur in air samples. Groupings of asbestos fibres and particles, referred to as "asbestos structures", are defined as fibre bundles, clusters and matrices. The numerical result of a TEM examination depends largely on whether the analyst assigns such an assemblage of fibres as a single entity, or as the estimated number of individual fibres which form the assemblage. It is therefore important that a logical system of counting criteria be defined, so that the interpretation of these complex structures is the same for all analysts, and so that the numerical result is meaningful. Imposition of specific structure-counting criteria generally requires that some interpretation, partially based on uncertain information on health effects, be made of each asbestos structure found. It is not the intention of this International Standard to make any interpretations based on health effects, and it is intended that a clear separation shall be made between recording of structure counting data, and later interpretation of those data. The system of coding specified in this International Standard permits a clear morphological description of the structures to be recorded in a concise manner suitable for later interpretation, if necessary, by a range of different criteria, without the necessity for re-examination of the specimens. In particular, the coding system is designed to permit the dimensions of each complex fibrous structure, and also whether these structures contain fibres longer than 5 µm, to be recorded. This approach permits later evaluations of the data to include considerations of particle respirability and comparisons with historical indices of asbestos exposure. Examples of the various types of morphological structure, and the manner in which these shall be recorded, are shown in figure C.1.

C.2 Structure definitions and treatment

Each fibrous structure that is a separate entity shall be designated as a primary structure. Each primary structure shall be designated as a fibre, bundle, cluster or matrix.

C.2.1 Fibre

Any particle with parallel or stepped sides, of minimum length 0,5 µm, and with an aspect ratio of 5/1 or greater, shall be defined as a fibre. For chrysotile asbestos, the single fibril shall be defined as a fibre. A fibre with stepped sides shall be assigned a width equal to the average of the minimum and maximum widths. This average shall be used as the width in determination of the aspect ratio.

C.2.2 Bundle

A grouping composed of apparently attached parallel fibres shall be defined as a bundle, with a width equal to an estimate of the mean bundle width, and a length equal to the maximum length of the structure. The overall aspect ratio of the bundle may have any value, provided that it contains individual constituent fibres with aspect ratios equal to or greater than 5/1. Bundles may exhibit diverging fibres at one or both ends.

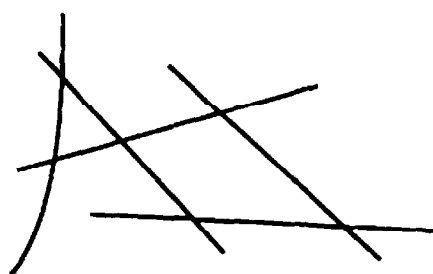
C.2.3 Cluster

An aggregate of two or more randomly oriented fibres, with or without bundles, shall be defined as a cluster. Clusters occur as two varieties.

C.2.3.1 disperse cluster (type D): Disperse and open network, in which at least one of the individual fibres or bundles can be separately identified and its dimensions measured;

C.2.3.2 compact cluster (type C): Complex and tightly bound network, in which one or both ends of each individual fibre or bundle is (are) obscured, such that the dimensions of individual fibres and bundles cannot be unambiguously determined.

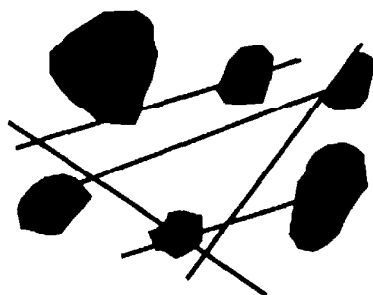
In practice, clusters can occur in which the characteristics of both types of cluster occur in the same structure. Where this occurs, the structure should be defined as a disperse cluster, and then a logical procedure should be followed by recording structure components according to the counting criteria. The procedure for treatment of clusters is illustrated by examples in figure C.2.



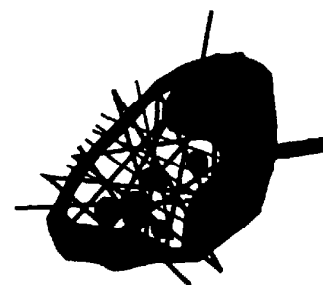
a) Disperse cluster (type D)



b) Compact cluster (type C)



c) Disperse matrix (type D)



d) Compact matrix (type C)

Figure C.1 — Fundamental morphological structure types

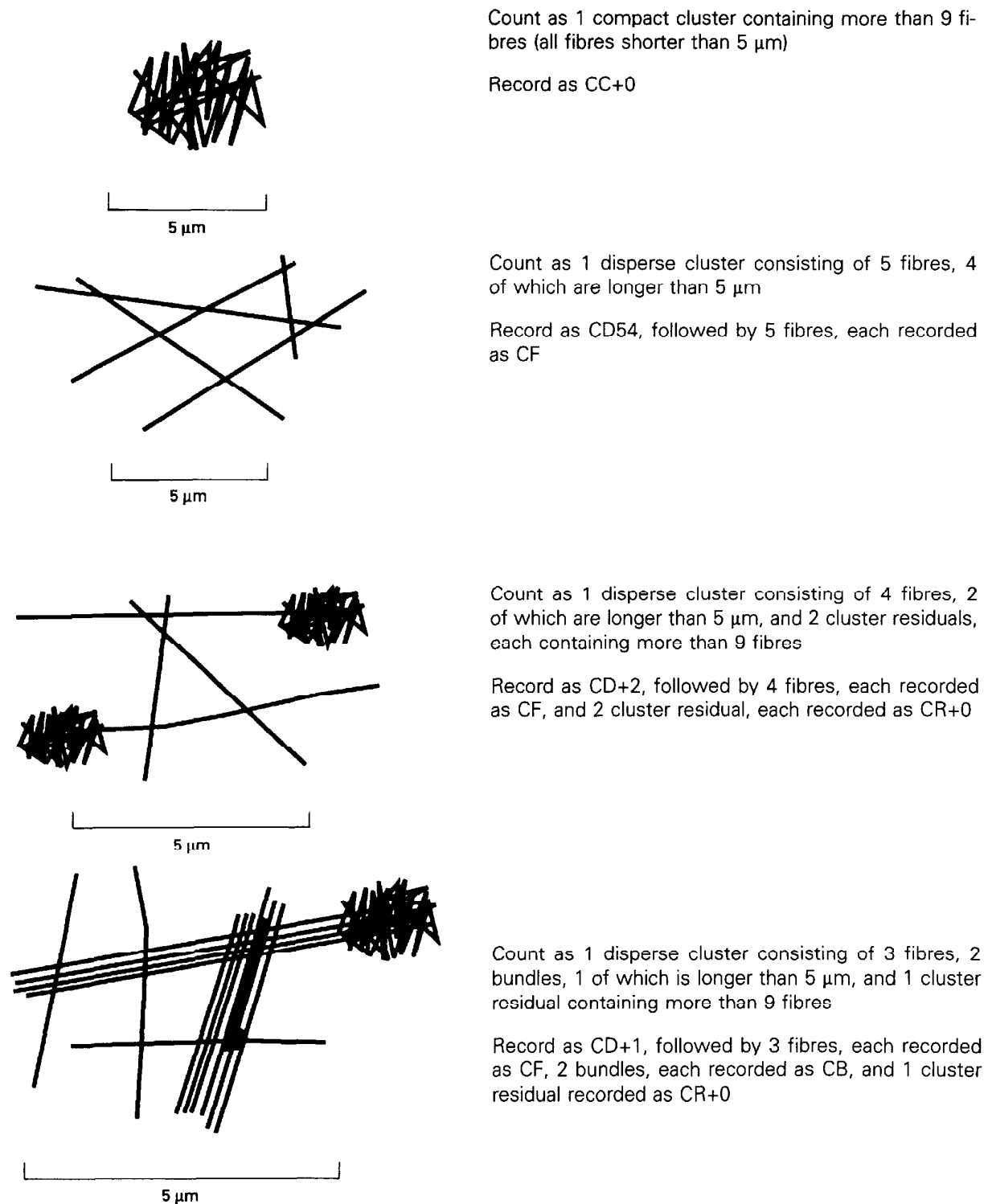


Figure C.2 — Examples of recording of complex asbestos clusters

C.2.4 Matrix

One or more fibres, or fibre bundles, may be attached to, or partially concealed by, a single particle or group of overlapping nonfibrous particles. This structure shall be defined as a matrix. The TEM image does not discriminate between particles which are attached to fibres, and those which have by chance overlapped in the TEM image. It is not known, therefore, whether such a structure is actually a complex particle, or whether it has arisen by a simple overlapping of particles and fibres on the filter.

Since a matrix structure may involve more than one fibre, it is important to define in detail how matrices shall be counted. Matrices exhibit different characteristics, and two types can be defined.

C.2.4.1 disperse matrix (type D): Structure consisting of a particle or linked group of particles, with overlapping or attached fibres or bundles in which at least one of the individual fibres or bundles can be separately identified and its dimensions measured.

C.2.4.2 compact matrix (type C): Structure consisting of a particle or linked group of particles, in which fibres or bundles can be seen either within the structure or projecting from it, such that the dimensions of individual fibres and bundles cannot be unambiguously determined.

In practice, matrices can occur in which the characteristics of both types of matrix occur in the same structure. Where this occurs, the structure should be assigned as a disperse matrix, and then a logical procedure should be followed by recording structure components according to the counting criteria. Examples of the procedure which shall be followed are shown in figure C.3.

C.2.5 Asbestos structure larger than 5 µm

Any fibre, bundle, cluster or matrix for which the largest dimension exceeds 5 µm. Asbestos structures larger than 5 µm do not necessarily contain asbestos fibres or bundles longer than 5 µm.

C.2.6 Asbestos fibre or bundle longer than 5 µm

An asbestos fibre of any width, or bundle of such fibres, which has a length exceeding 5 µm.

C.2.7 PCM equivalent structure

Any fibre, bundle, cluster or matrix with an aspect ratio of 3/1 or greater, longer than 5 µm, and which has a diameter between 0,2 µm and 3,0 µm. PCM equivalent structures do not necessarily contain fibres or bundles longer than 5 µm, or PCM equivalent fibres.

C.2.8 PCM equivalent fibre

Any particle with parallel or stepped sides, with an aspect ratio of 3/1 or greater, longer than 5 µm, and which has a diameter between 0,2 µm and 3,0 µm. For chrysotile, PCM equivalent fibres will always be bundles.

C.3 Other structure counting criteria

C.3.1 Structures which intersect grid bars

A structure which intersects a grid bar shall only be counted on two sides of the grid opening, as illustrated in figure C.4. Record the dimensions of the structure such that the obscured portions of components are taken to be equivalent to the unobscured portions, as shown by the broken lines in figure C.4. For example, the length of a fibre intersecting a grid bar is taken to be twice the unobscured length. Structures intersecting either of the other two sides shall not be included in the count.

C.3.2 Fibres which extend outside the field of view

During scanning of a grid opening, count fibres which extend outside the field of view systematically, so as to avoid double-counting. In general, a rule should be established so that fibres extending outside the field of view in only two quadrants are counted. The procedure is illustrated by figure C.5. Measure the length of each of these fibre by moving the specimen to locate the other end of the fibre, and then return to the original field of view before continuing to scan the specimen. Fibres without terminations within the field of view shall not be counted.

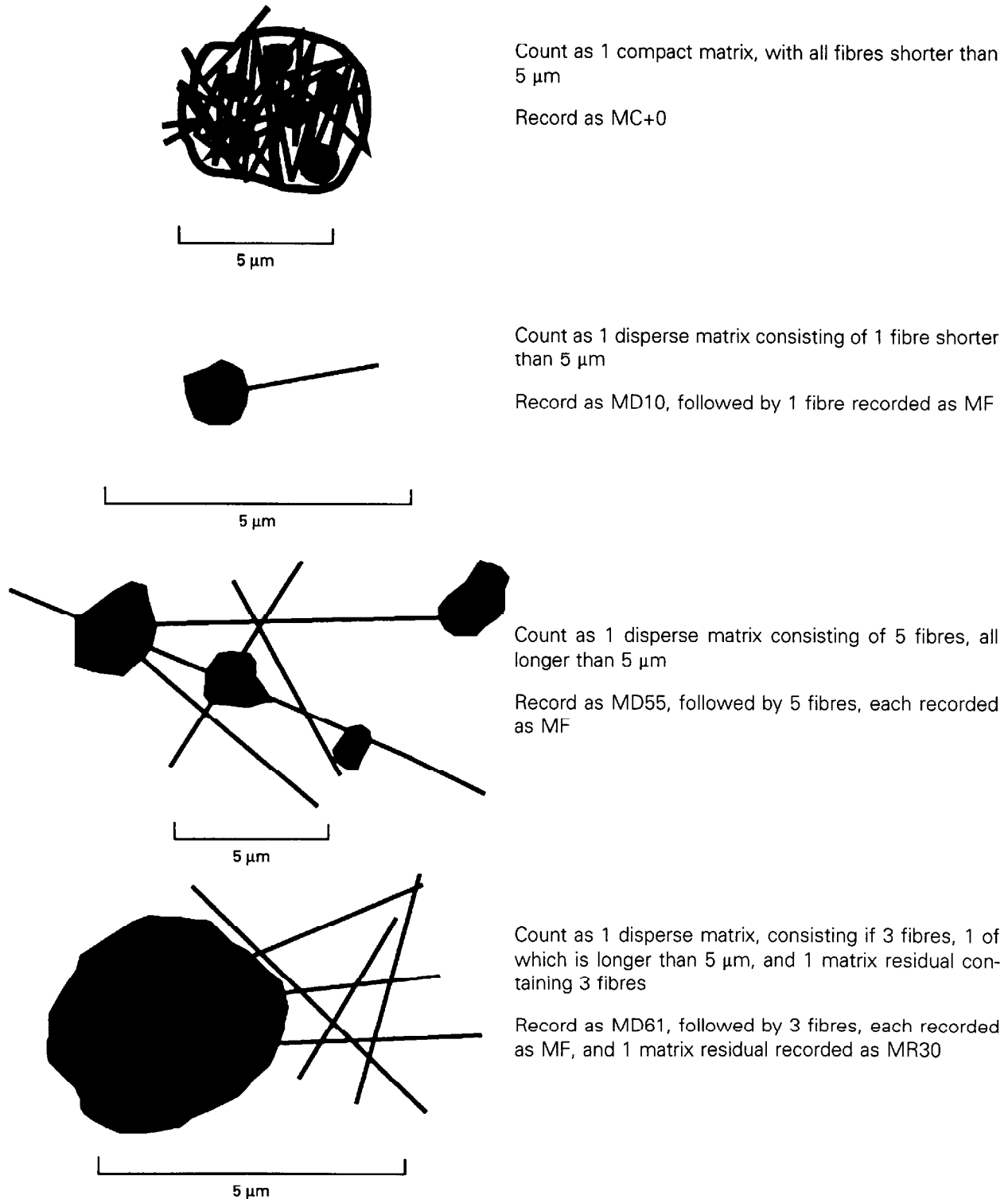


Figure C.3 — Examples of recording of complex asbestos matrices

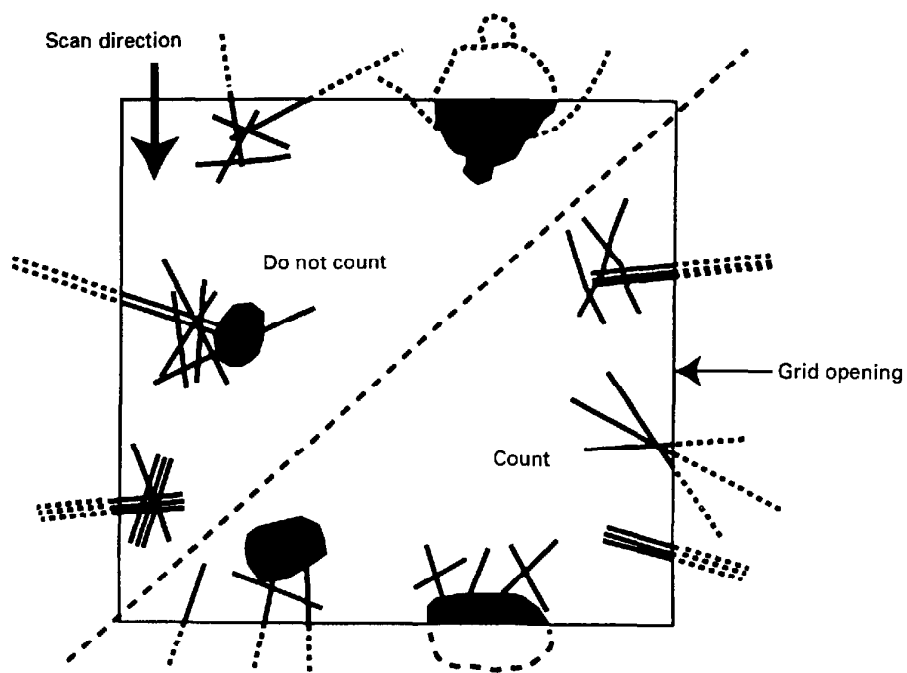


Figure C.4 — Example of counting of structures which intersect grid bars

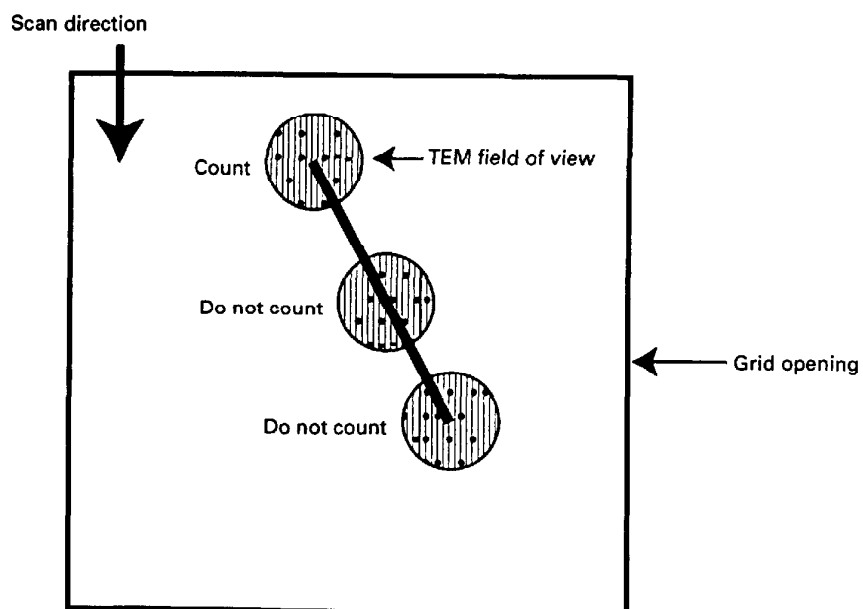


Figure C.5 — Example of counting of fibres which extend outside the field of view

C.4 Procedure for data recording

C.4.1 General

The morphological codes specified are designed to facilitate computer data processing, and to allow recording of a complete representation of the important features of each asbestos structure. The procedure requires that the microscopist classify each primary fibrous structure into one of the four fundamental categories: fibres, bundles, clusters and matrices.

C.4.2 Fibres

On the structure counting form, a fibre as defined in C.2.1 shall be recorded by the designation "F". If the fibre is a separately-counted part of a cluster or matrix, the fibre shall be recorded by the designation "CF", or "MF", depending on whether it is a component of a cluster or matrix.

C.4.3 Bundles

On the structure counting form, a bundle as defined in C.2.2 shall be recorded by the designation "B". If the bundle is a separately-counted part of a cluster or matrix, the bundle shall be recorded by the designation "CB", or "MB", depending on whether it is a component of a cluster or matrix.

C.4.4 Disperse clusters (type D)

On the structure counting form, an isolated cluster of type D as defined in C.2.3 shall be recorded by the designation "CD", followed by a two-digit number. The first digit represents the analyst's estimate of the total number of fibres and bundles comprising the structure. The digit shall be from 1 to 9, or designated as "+" if there are estimated to be more than 9 component fibres or bundles. The second digit shall represent, in the same manner, the total number of fibres and bundles longer than 5 µm contained in the structure. The overall dimensions of the cluster, in two perpendicular directions representing the maximum dimensions, shall be recorded. In order of decreasing length, up to 5 component fibres or bundles shall be separately recorded, using the codes "CF" (cluster fibre) and "CB" (cluster bundle). If, after accounting for prominent component fibres and bundles, a group of clustered fibres remains, this shall be recorded by the designation "CR" (cluster residual). If the remaining clustered fibres are present as more than one localized group, it may be necessary to record more than one cluster residual. Do not record more than 5 cluster residuals for any cluster. A cluster residual shall be measured and assigned a two-digit

number, derived in the same manner as specified for the overall cluster. Optionally, if the number of component fibres and bundles in either the original cluster or the cluster residual is outside the range 1 – 9, additional information concerning the number of component fibres and bundles may be noted in the "comments" column.

C.4.5 Compact clusters (type C)

On the structure counting form, an isolated cluster of type C as defined in C.2.3 shall be recorded by the designation "CC", followed by a two-digit number. The two-digit number describing the numbers of component fibres and bundles shall be assigned in the same manner as for clusters of type D. The overall dimensions of the cluster in two perpendicular directions shall be recorded in the same manner as for clusters of type D. By definition, the constituent fibres and bundles of compact clusters cannot be separately measured; therefore, no separate tabulation of component fibres or bundles can be made.

C.4.6 Disperse matrices (type D)

On the structure counting form, an isolated matrix of type D as defined in C.2.4 shall be recorded by the designation "MD", followed by a two-digit number. The two-digit number shall be assigned in the same manner as for clusters of type D. The overall dimensions of the matrix in two perpendicular directions shall be recorded in the same manner as for clusters of type D. In order of decreasing length, up to 5 component fibres or bundles shall be separately recorded, using the codes "MF" (matrix fibre) and "MB" (matrix bundle). If after accounting for prominent component fibres and bundles, matrix material containing asbestos fibres remains, this shall be recorded by the designation "MR" (matrix residual). If the remaining matrix fibres are present as more than one localized group, it may be necessary to record more than one matrix residual. Do not record more than 5 matrix residuals for any matrix. A matrix residual shall be measured and assigned a two-digit number, derived in the same manner as specified for the overall matrix. Optionally, if the number of component fibres or bundles in either the original matrix or the matrix residual is outside the range 1 – 9, additional information concerning the number of component fibres and bundles may be noted in the "comments" column.

C.4.7 Compact matrices (type C)

On the structure counting form, an isolated matrix of type C as defined in C.2.4 shall be recorded by the

designation "MC", followed by a two-digit number. The two-digit number shall be assigned in the same manner as for clusters of type D. The overall dimensions of the matrix in two perpendicular directions shall be recorded in the same manner as for clusters of type D. By definition, the constituent fibres and bundles of compact matrices cannot be separately measured; therefore, no separate tabulation of component fibres or bundles can be made.

C.4.8 Procedure for recording of partially obscured fibres and bundles

The proportion of the length of a fibre or bundle that is obscured by other particulates shall be used as the basis for determining whether a fibre or bundle is to be recorded as a separate component or is to be considered as a part of a matrix of type C or part of a matrix residual. If the obscured length could not possibly be more than one-third of the total length, the fibre or bundle shall be considered a prominent feature to be separately recorded. The assigned length for each such partially obscured fibre or bundle shall be equal to the visible length plus the maximum possible contribution from the obscured portion. Fibres or bundles which appear to cross the matrix, and for which both ends can be located approximately, shall be included in the maximum of 5 and recorded according to the counting criteria as separate fibres or bundles. If the obscured length could be more than one third of the total length, the fibre or bundle shall

be considered as a part of a compact matrix of type C or part of a matrix residual.

C.5 Special considerations for counting of PCM equivalent structures

Use 3/1 as the minimum aspect ratio for counting of PCM equivalent structures. This aspect ratio definition is required in order to achieve comparability of the results for this size range of structure with historical optical measurements, but use of this aspect ratio definition does not significantly affect the ability to interpret the whole fibre size distribution in terms of a minimum 5/1 aspect ratio. Some applications may require that a count be made of PCM equivalent structures only. The coding system permits discrimination between PCM equivalent structures that contain fibres and bundles longer than 5 µm and those that do not.

NOTE 16 In general, clusters and matrices will yield fewer components as the minimum dimensions specified for countable fibres are increased. Thus, it may be found that a particular structure yields a higher number of component fibres and bundles in a count for all fibre sizes than it does at a reduced magnification when only fibres and bundles longer than 5 µm are being counted. However, the requirement that component fibres and bundles be recorded in decreasing length order ensures that the data are consistent for a particular structure, regardless of the size category of fibres being counted and the magnification in use.

Annex D (normative)

Fibre identification procedure

D.1 General

The criteria used for identification of asbestos fibres may be selected depending on the intended use of the measurements. In some circumstances, there can be a requirement that fibres shall be unequivocally identified as a specific mineral species. In other circumstances, there can be sufficient knowledge about the sample, so that rigorous identification of each fibre need not be carried out. The time required to perform the analysis, and therefore the cost of analysis, can vary widely depending on the identification criteria considered which are to be sufficiently definitive. The combination of criteria considered definitive for identification of fibres in a particular analysis shall be specified before the analysis is made, and this combination of criteria shall be referred to as the "level" of analysis. Various factors related to instrumental limitations and the character of the sample may prevent satisfaction of all of the specified fibre identification criteria for a particular fibre. Therefore, a record shall be made of the identification criteria which were satisfied for each suspected asbestos fibre included in the analysis. For example, if both ED and EDXA were specified to be attempted for definitive identification of each fibre, fibres with chrysotile morphology which, for some reason, do not give an ED pattern but which do yield an EDXA spectrum corresponding to chrysotile, are categorized in a way which conveys the level of confidence to be placed in the identification.

D.2 ED and EDXA techniques

D.2.1 General

Initially, fibres are classified into two categories on the basis of morphology: those fibres with tubular morphology, and those fibres without tubular morphology. Further analysis of each fibre is conducted using ED and EDXA methods. The following procedures should be used when fibres are examined by ED and EDXA.

The crystal structures of some mineral fibres, such as chrysotile, are easily damaged by the high current densities required for EDXA examination. Therefore,

investigation of these sensitive fibres by ED should be completed before attempts are made to obtain EDXA spectra from the fibres. When more stable fibres, such as the amphiboles, are examined, EDXA and ED may be used in either order.

D.2.2 ED techniques

The ED technique can be either qualitative or quantitative. Qualitative ED consists of visual examination, without detailed measurement, of the general characteristics of the ED pattern obtained on the TEM viewing screen from a randomly oriented fibre. ED patterns obtained from fibres with cylindrical symmetry, such as chrysotile, do not change when the fibres are tilted about their axes, and patterns from randomly oriented fibres of these minerals can be interpreted quantitatively. For fibres which do not have cylindrical symmetry, only those ED patterns obtained when the fibre is oriented with a principal crystallographic axis closely parallel with the incident electron beam direction can be interpreted quantitatively. This type of ED pattern shall be referred to as a "zone-axis ED pattern". In order to interpret a zone-axis ED pattern quantitatively, it shall be recorded photographically and its consistency with known mineral structures shall be checked. A computer program may be used to compare measurements of the zone-axis ED pattern with corresponding data calculated from known mineral structures. The zone-axis ED pattern obtained by examination of a fibre in a particular orientation can be insufficiently specific to permit unequivocal identification of the mineral fibre, but is often possible to tilt the fibre to another angle and to record a different ED pattern corresponding to another zone-axis. The angle between the two zone-axes can also be checked for consistency with the structure of a suspected mineral.

For visual examination of the ED pattern, the camera length of the TEM should be set to a low value of approximately 250 mm and the ED pattern should then be viewed through the binoculars. This procedure minimizes the possible degradation of the fibre by the electron irradiation. However, the pattern is distorted by the tilt angle of the viewing screen. A camera length of at least 2 m should be used when

the ED pattern is recorded, if accurate measurement of the pattern is to be possible. It is necessary that, when obtaining an ED pattern to be evaluated visually or to be recorded, the sample height shall be properly adjusted to the eucentric point and the image shall be focussed in the plane of the selected area aperture. If this is not done, there may be some components of the ED pattern which do not originate from the selected area. In general, it will be necessary to use the smallest available ED aperture.

For accurate measurements of the ED pattern, an internal calibration standard shall be used. A thin coating of gold, or another suitable calibration material, shall be applied to the underside of the TEM specimen. This coating may be applied either by vacuum evaporation or, more conveniently, by sputtering. The polycrystalline gold film yields diffraction rings on every ED pattern and these rings provide the required calibration information.

To form an ED pattern, move the image of the fibre to the centre of the viewing screen, adjust the height of the specimen to the eucentric position, and insert a suitable selected area aperture into the electron beam so that the fibre, or a portion of it, occupies a large proportion of the illuminated area. The size of the aperture and the portion of the fibre shall be such that particles other than the one to be examined are excluded from the selected area. Observe the ED pattern through the binoculars. During the observation, the objective lens current should be adjusted to the point where the most complete ED pattern is obtained. If an incomplete ED pattern is still obtained, move the particle around within the selected area to attempt to optimize the ED pattern, or to eliminate possible interferences from neighbouring particles.

If a zone-axis ED analysis is to be attempted on the fibre, the sample shall be mounted in the appropriate holder. The most convenient holder allows complete rotation of the specimen grid and tilting of the grid about a single axis. Rotate the sample until the fibre image indicates that the fibre is oriented with its length coincident with the tilt axis of the goniometer, and adjust the sample height until the fibre is at the eucentric position. Tilt the fibre until an ED appears which is a symmetrical, two dimensional array of spots. The recognition of zone-axis alignment conditions requires some experience on the part of the operator. During tilting of the fibre to obtain zone-axis conditions, the manner in which the intensities of the spots vary should be observed. If weak reflections

occur at some points on a matrix of strong reflections, the possibility of twinning or multiple diffraction exists, and some caution should be exercised in the selection of diffraction spots for measurement and interpretation. A full discussion of electron diffraction and multiple diffraction can be found in the references by J.A. Gard [11] P.B. Hirsch *et al* [14] and H.R. Wenck [42] included in annex J. Not all zone-axis patterns which can be obtained are definitive. Only those which have closely spaced reflections corresponding to low indices in at least one direction should be recorded. Patterns in which all d-spacings are less than about 0,3 nm are not definitive. A useful guideline is that the lowest angle reflections should be within the radius of the first gold diffraction ring (111), and that patterns with smaller distances between reflections are usually the most definitive.

Five spots, closest to the centre spot, along two intersecting lines of the zone-axis pattern shall be selected for measurement, as shown in figure D.1. The distances of these spots from the centre spot and the four angles shown provide the required data for analysis. Since the centre spot is usually very overexposed, it does not provide a well-defined origin for these measurements. The required distances shall therefore be obtained by measuring between pairs of spots symmetrically disposed about the centre spot, preferably separated by several repeat distances. The distances shall be measured with a precision of better than 0,3 mm, and the angles to a precision of better than 2,5°. The diameter of the first or second ring of the calibration pattern (111 and 200) shall also be measured with a precision of better than 0,3 mm.

Using gold as the calibration material, the radius-based camera constant is given by

$$\lambda L = 0,117\ 74D\ \text{mm}\cdot\text{nm}\ (\text{first ring})$$

$$\lambda L = 0,101\ 97D\ \text{mm}\cdot\text{nm}\ (\text{second ring})$$

D.2.3 EDXA measurements

Interpretation of the EDXA spectrum may be either qualitative or quantitative. For qualitative interpretation of a spectrum, the X-ray peaks originating from the elements in the fibre are recorded. For quantitative interpretation, the net peak areas, after background subtraction, are obtained for the X-ray peaks originating from the elements in the fibre. This method provides quantitative interpretation for those minerals which contain silicon.

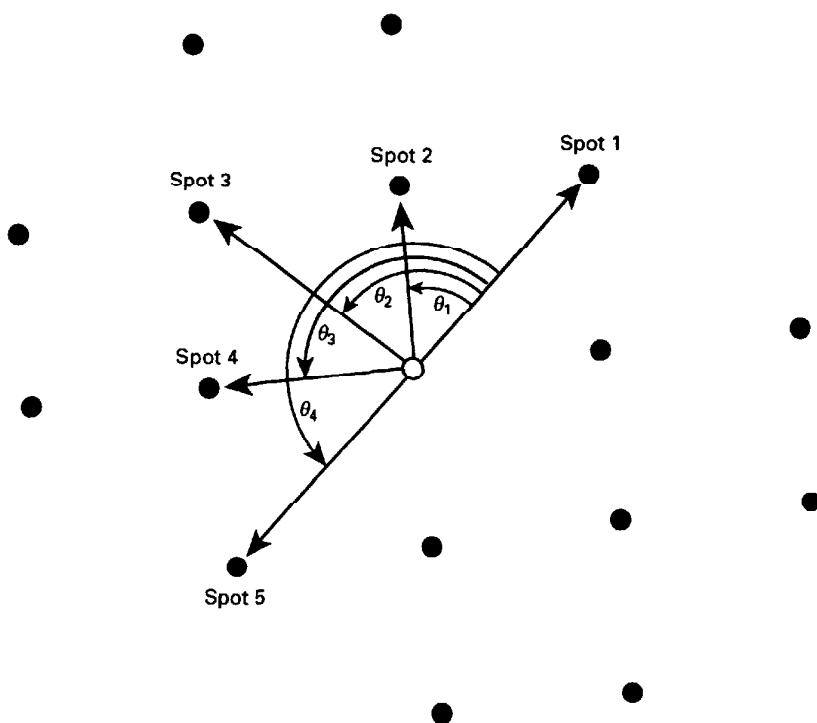


Figure D.1 — Example of measurement of zone-axis SAED patterns

To obtain an EDXA spectrum, move the image of the fibre to the centre of the screen and remove the objective aperture. Select an appropriate electron beam diameter and deflect the beam so that it impinges on the fibre. Depending on the instrumentation, it may be necessary to tilt the specimen towards the X-ray detector and, in some instruments, to use the Scanning Transmission Electron Microscopy (STEM) mode of operation.

The time for acquisition of a suitable spectrum varies with the fibre diameter, and also with instrumental factors. For quantitative interpretation, spectra should have a statistically valid number of counts in each peak. Analyses of small diameter fibres which contain sodium are the most critical, since it is in the low energy range that the X-ray detector is least sensitive. Consequently, it is necessary to acquire a spectrum for a period that is sufficiently long for the sodium to be detected in such fibres. It has been found that satisfactory quantitative analyses can be obtained if acquisition is continued until the background subtracted silicon $K\alpha$ peak integral exceeds 10 000 counts. The spectrum should then be manipulated to subtract the background and to obtain the net areas of the elemental peaks.

After quantitative EDXA classification of some fibres by computer analysis of the net peak areas, it may be possible to classify further fibres in the same sample

on the basis of comparison of spectra at the instrument. Frequently, visual comparisons can be made after somewhat shorter acquisition times.

D.3 Interpretation of fibre analysis data

D.3.1 Chrysotile

The morphological structure of chrysotile is characteristic, and with experience, can be recognized readily. However, a few holder minerals have a similar appearance, and morphological observation by itself is inadequate for most samples. The ED pattern obtained from chrysotile is quite specific for this mineral if the specified characteristics of the pattern correspond to those from reference chrysotile. However, depending on the past history of the fibre, and on a number of other factors, the crystal structure of a particular fibre may be damaged, and it may not yield an ED pattern. In this case, the EDXA spectrum may be the only data available to supplement the morphological observations.

D.3.2 Amphiboles

Since the fibre identification procedure for asbestos fibres other than chrysotile can be involved and time-consuming, computer programmes, such as that developed by B.L. Rhoades (see annex J, reference

[32]), are recommended for interpretation of zone-axis ED patterns. The published literature contains composition and crystallographic data for all of the fibrous minerals likely to be encountered in TEM analysis of air samples, and the compositional and structural data from the unknown fibre should be compared with the published data. Demonstration that the measurements are consistent with the data for a particular test mineral does not uniquely identify the unknown, since the possibility exists that data from other minerals may also be consistent. It is, however, unlikely that a mineral of another structural class could yield data consistent with that from an amphibole fibre identified by quantitative EDXA and two zone-axis ED patterns.

Suspected amphibole fibres should be classified initially on the basis of chemical composition. Either qualitative or quantitative EDXA information may be used as the basis for this classification. From the published data on mineral compositions, a list of minerals which are consistent in composition with that measured for the unknown fibre should be compiled. To proceed further, it is necessary to obtain the first zone-axis ED pattern, according to D.2.2.

It is possible to specify a particular zone-axis pattern for identification of amphibole, since a few patterns are often considered to be characteristic. Unfortunately, for a fibre with random orientation on a TEM grid, no specimen holder and goniometer currently available will permit convenient and rapid location of two preselected zone-axes. The most practical approach has been adopted, which is to accept those low index patterns which are easily obtained, and then to test their consistency with the structures of the minerals already preselected on the basis of the EDXA data. Even the structures of non-amphibole minerals in this preselected list shall be tested against the zone-axis data obtained for the unknown fibre, since non-amphibole minerals in some orientations may yield similar patterns consistent with amphibole structures.

The zone-axis ED interpretation shall include all minerals previously selected from the mineral data file as being chemically compatible with the EDXA data. This procedure will usually shorten the list of minerals for which solutions have been found. A second set of zone-axis data from another pattern obtained on the

same fibre can then be processed, either as further confirmation of the identification, or to attempt elimination of an ambiguity. In addition, the angle measured between the orientations of the two zone-axes can be checked for consistency with the structures of the minerals. Caution should be exercised in rationalizing the inter-zone-axis angle, since if the fibre contains \vec{c} -axis twinning, the two zone-axis ED patterns may originate from the separate twin crystals. In practice, the full identification procedure will normally be applied to very few fibres, unless precise identification of all fibres is required for a particular reason.

D.4 Fibre classification categories

It is not always possible to proceed to a definitive identification of a fibre; this may be due to instrumental limitations or to the actual nature of the fibre. In many analyses, a definitive identification of each fibre may not actually be necessary if there is other knowledge available about the sample, or if the concentration is below a level of interest. The analytical procedure shall therefore take into account both instrumental limitations and varied analytical requirements. Accordingly, a system for fibre classification is used to permit accurate recording of data. The classifications are shown in tables D.1 and D.2, and are directed towards identification of chrysotile and amphibole respectively. Fibres shall be reported in these categories.

The general principle to be followed in this analytical procedure is first to define the most specific fibre classification which is to be attempted, or the "level" of analysis to be conducted. Then, for each fibre examined, record the classification which is actually achieved. Depending on the intended use of the results, criteria for acceptance of fibres as "identified" can then be established at any time after completion of the analysis.

In an unknown sample, chrysotile will be regarded as confirmed only if a recorded, calibrated ED pattern from one fibre in the CD categories is obtained, or if measurements of the ED pattern are recorded at the instrument. Amphibole will be regarded as confirmed only by obtaining recorded data which indicates exclusively the presence of amphiboles for fibres classified in the AZQ, AZZ or AZZQ categories.

Table D.1 — Classification of fibres with tubular morphology

Category	Description
TM	Tubular Morphology, not sufficiently characteristic for classification as chrysotile
CM	Characteristic Chrysotile Morphology
CD	Chrysotile SAED pattern
CQ	Chrysotile composition by Quantitative EDXA
CMQ	Chrysotile Morphology and composition by Quantitative EDXA
CDQ	Chrysotile SAED pattern and composition by Quantitative EDXA
NAM	Non-Asbestos Mineral

Table D.2 — Classification of fibres without tubular morphology

Category	Description
UF	Unidentified Fibre
AD	Amphibole by random orientation SAED (shows layer pattern of 0,53 nm spacing)
AX	Amphibole by qualitative EDXA. Spectrum has elemental components consistent with amphibole
ADX	Amphibole by random orientation SAED and qualitative EDXA
AQ	Amphibole by Quantitative EDXA
AZ	Amphibole by one Zone-axis SAED pattern
ADQ	Amphibole by random orientation SAED and Quantitative EDXA
AZQ	Amphibole by one Zone-axis SAED pattern and Quantitative EDXA
AZZ	Amphibole by two Zone-axis SAED patterns, with consistent interaxial angle
AZZQ	Amphibole by two Zone-axis SAED patterns, with consistent interaxial angle, and Quantitative EDXA
NAM	Non-Asbestos Mineral

D.4.1 Procedure for classification of fibres with tubular morphology suspected to be chrysotile

Occasionally, fibres are encountered which have tubular morphology similar to that of chrysotile, but which cannot be characterized further either by ED or EDXA. They may be non-crystalline, in which case ED techniques are not useful, or they may be in a position on the grid which does not permit an EDXA spectrum to be obtained. Alternatively, the fibre may be of organic origin, but the morphology and composition may not be sufficiently definitive enough to be disregarded. Accordingly, there is a requirement to record each fibre, and to specify how confidently each fibre can be identified. Classification of fibres will

meet with various degrees of success. Figure D.2 shows the classification procedure to be used for fibres which display any tubular morphology. The chart is self explanatory, and every fibre is either rejected as a non-asbestos mineral (NAM), or classified in some way which by some later criterion could still contribute to the chrysotile fibre count.

Morphology is the first consideration, and if this is not similar to that usually seen in chrysotile standard samples, designate the initial classification as TM. Regardless of the doubtful morphology, examine the fibre by ED and EDXA methods according to figure D.2. Where the morphology is more definitive, it may be possible to classify the fibre as having chrysotile morphology (CM).

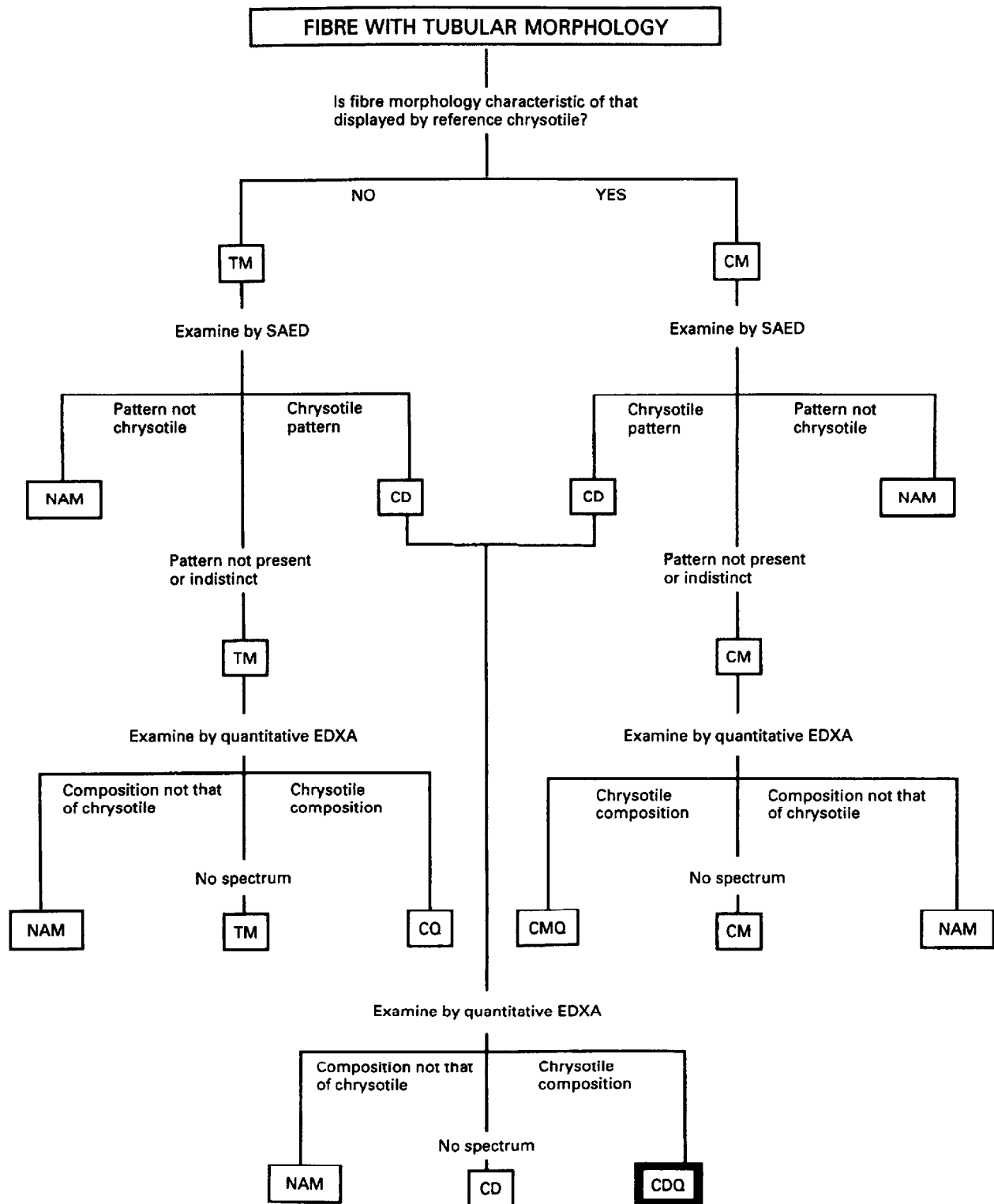


Figure D.2 — Classification chart for fibre with tubular morphology

For classification as CM, the morphological characteristics required are the following:

- a) the individual fibrils should have high aspect ratios exceeding 5/1, and be about 30 nm to 40 nm in diameter;
- b) the electron scattering power of the fibre at 60 kV to 100 kV accelerating potential should be sufficiently low for the internal structure to be visible;
- c) there should be some evidence of an internal structure suggesting a tubular appearance similar to that shown by reference UICC chrysotile, which may degrade in the electron beam.

Examine every fibre having these morphological characteristics by the ED technique, and classify as chrysotile by ED (CD) only those which give diffraction patterns with the precise characteristics shown in figure D.3. The relevant features in this pattern for identification of chrysotile are as follows:

- a) the (002) reflections should be examined to determine that they correspond closely to a spacing of 0,73 nm;
- b) the layer line repeat distance should correspond to 0,53 nm;
- c) there should be "streaking" of the (110) and (130) reflections.

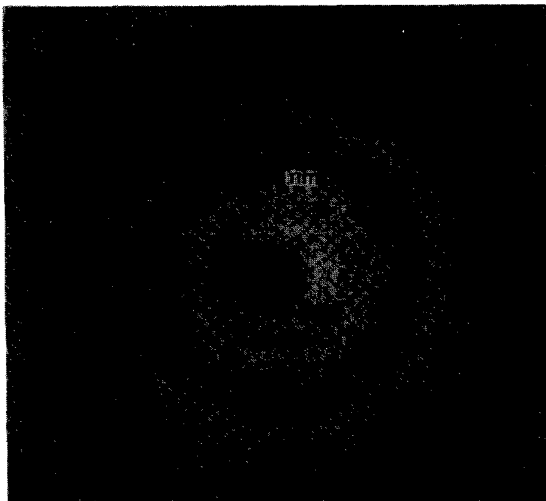


Figure D.3 — Chrysotile SAED pattern

Using the millimetre calibrations on the TEM viewing screen, these observations can readily be made at the instrument. If documentary proof of fibre identification is required, record a TEM micrograph of at least one representative fibre, and record its ED pattern on a separate film or plate. This film or plate shall also carry calibration rings from a known polycrystalline substance such as gold. This calibrated pattern is the only documentary proof that the particular fibre is chrysotile, and not some other tubular or scrolled species such as halloysite, palygorskite, talc or vermiculite. The proportion of fibres which can be successfully identified as chrysotile by ED is variable, and to some extent dependent on both the instrument and the procedures of the operator. The fibres that fail to yield an identifiable ED pattern will remain in the TM or CM categories unless they are examined by EDXA.

In the EDXA analysis of chrysotile there are only two elements which are relevant. For fibre classification, the EDXA analysis shall be quantitative. If the spectrum displays prominent peaks from magnesium and silicon, with their areas in the appropriate ratio, and with only minor peaks from other elements, classify the fibre as chrysotile by quantitative EDXA, in the categories CQ, CMQ, or CDQ, as appropriate.

D.4.2 Procedure for classification of fibres without tubular morphology, suspected to be amphibole

Every particle without tubular morphology and which is not obviously of biological origin, with an aspect ratio of 5/1 or greater, and having parallel or stepped sides, shall be considered as a suspected amphibole fibre. Further examination of the fibre by ED and EDXA techniques will meet with a variable degree of success, depending on the nature of the fibre and on a number of instrumental limitations. It will not be possible to identify every fibre completely, even if time and cost are of no concern. Moreover, confirmation of the presence of amphibole can be achieved only by quantitative interpretation of zone-axis ED patterns, a very time-consuming procedure. Accordingly, for routine samples from unknown sources, this analytical procedure limits the requirement for zone-axis ED work to a minimum of one fibre representative of each compositional class reported. In some samples, it may be necessary to identify more fibres by the zone-axis technique. When analysing samples from well-characterized sources, the cost of identification by zone-axis methods may not be justified.

The 0,53 nm layer spacing of the random orientation ED pattern is not by itself diagnostic for amphibole. However, the presence of c^* -axis twinning in many fi-

bres leads to contributions to the layers in the patterns by several individual parallel crystals of different axial orientations. This apparently random positioning of the spots along the layer lines, if also associated with a high fibre aspect ratio, is a characteristic of amphibole asbestos, and thus has some limited diagnostic value. If a pattern of this type is not obtained, the identity of the fibre is still ambiguous, since the absence of a recognizable pattern may be a consequence of an unsuitable orientation relative to the electron beam, or the fibre may be some other mineral species.

Figure D.4 shows the fibre classification chart to be used for suspected amphibole fibres. This chart shows all the classification paths possible in analysis of a suspected amphibole fibre, when examined systematically by ED and EDXA. Two routes are possible, depending on whether an attempt to obtain an EDXA spectrum or a random orientation ED pattern is made first. The normal procedure for analysis of a sample of unknown origin will be to examine the fibre by random orientation ED, qualitative EDXA, quantitative EDXA, and zone-axis ED, in this sequence. The final fibre classification assigned will be defined either by successful analysis at the maximum required level,

or by the instrumental limitations. Any instrumental limitations which affect the quality of the results shall be noted. Record the maximum classification achieved for each fibre on the counting sheet in the appropriate column. The various classification categories can then be combined later in any desired way for calculation of the fibre concentration. The complete record of the results obtained when attempting to identify each fibre can also be used to re-assess the data if necessary.

In the unknown sample, zone-axis analysis will be required if the presence of amphibole is to be unequivocally confirmed. For this level of analysis, attempt to raise the classification of every suspected amphibole fibre to the ADQ category by inspection of the random orientation ED pattern and the EDXA spectrum. In addition, examine at least one fibre from each type of suspected amphibole found by zone-axis methods to confirm their identification. In most cases, because information exists about possible sources of asbestos in close proximity to the air sampling location, some degree of ambiguity of identification can be accepted. Lower levels of analysis can therefore be accepted for these situations.

Annex E

(normative)

Determination of the concentrations of asbestos fibres and bundles longer than 5 µm, and PCM equivalent asbestos fibres

In order to provide increased statistical precision and improved analytical sensitivity for those asbestos fibres and bundles longer than 5 µm, it may be decided to perform additional fibre counting at a lower magnification, taking account only into fibres and bundles within this dimensional range. The result shall be specified as "number of asbestos fibres and bundles longer than 5 µm". For this examination, use a magnification of approximately $\times 10\,000$, and continue to assign a morphological code to each structure according to the procedures specified in annex C. Record fibres and bundles only if their lengths exceed 5 µm. Record cluster and matrix components only if their lengths exceed 5 µm.

It may also be decided to provide increased statistical precision and improved analytical sensitivity for fibrous structures longer than 5 µm, with diameters between 0,2 µm and 3,0 µm, which have historically been the basis of risk estimation in the occupational environment (PCM equivalent asbestos fibres). Use a magnification of approximately $\times 5\,000$ for this extended fibre count. The result shall be specified as "number of PCM equivalent asbestos fibres". Asbestos structures within this dimensional range do not necessarily incorporate asbestos fibres or bundles longer than 5 µm.

Continue the extended sample examination until 100 asbestos structures have been counted, or until a sufficient area of the specimen has been examined to achieve the desired analytical sensitivity calculated according to table 1. The grid openings examined shall be divided approximately equally between a minimum of two specimen grids.

NOTES

17 The specimen area corresponding to the area of filter examined in the PCM fibre counting methods is 0,785 mm², and is equivalent to approximately 100 grid openings of a 200 mesh grid.

18 Some National Standards require that asbestos fibres longer than 2,5 µm, with diameters between 0,2 µm and 3,0 µm be counted. Use a magnification of $\times 5\,000$ for counting fibres within these dimensional ranges.

19 The minimum aspect ratio for definition of a fibre in PCM fibre counting methods and in some National Standards is 3/1. Use of a 3/1 aspect ratio is permitted in this International Standard, if this aspect ratio is mentioned in the test report.

The test reports shall include all of the items listed in clause 11.

Annex F (normative)

Calculation of results

F.1 General

The results should be calculated using the procedures specified below. The results can be conveniently calculated using a computer programme.

F.2 Test for uniformity of distribution of fibrous structures on TEM grids

A check shall be made using the chi-square test, to determine whether the asbestos structures found on individual grid openings are randomly and uniformly distributed among the grid openings. If the total number found in k grid openings is n , and the areas of the k individual grid openings are designated A_1 to A_k , then the total area of TEM specimen examined is

$$A = \sum_{i=1}^{i=k} A_i$$

The fraction of the total area examined which is represented by the individual grid opening area, p_i , is given by A_i/A . If the structures are randomly and uniformly dispersed over the k grid openings examined, the expected number of structures falling in one grid opening with area A_i is np_i . If the observed number of structures found on that grid opening is n_i , then

$$\chi^2 = \sum_{i=1}^{i=k} \frac{(n_i - np_i)^2}{np_i}$$

This value shall be compared with significance points of the chi-square distribution, having $(k - 1)$ degrees of freedom. Significance levels lower than 0,1 % may be cause for the sample analysis to be rejected, since this correspond to a very inhomogeneous deposit. If the structure count fails this test, the precision of the result will be uncertain, and if new air samples cannot be collected, additional grid openings may be examined or the sample may be prepared by an indirect method.

F.3 Calculation of the analytical sensitivity

Calculate the required analytical sensitivity S , expressed in number of structures per litre, using the following equation:

$$S = \frac{A_f}{kA_gV}$$

where

- A_f is the area, in square millimetres, of sample collection filter;
- A_g is the area, in square millimetres, of TEM specimen grid opening;
- k is the number of grid openings examined;
- V is the volume of air sampled, in litres.

F.4 Calculation of the mean and confidence interval of the structure concentration

In the structure count made according to this International Standard, a number of grid openings have been sampled from a population of grid openings, and it is required to determine the mean grid opening structure count for the population on the basis of this small sample. The interval about the sample mean which, with 95 % confidence, contains the population mean, is also required.

F.4.1 Calculation of the mean structure concentration

Calculate the mean structure concentration C , expressed in number of structures per litre, using the following equation:

$$C = Sn$$

where

- S is the analytical sensitivity, expressed in number of structures per litre;
- n is the total number of structures found on all grid openings examined.

F.4.2 Calculation of confidence intervals

The distribution of structures on the grid openings should theoretically approximate to a Poisson distribution. Because of fibre aggregation and size-dependent identification effects, the actual structure counting data often does not conform to the Poisson distribution, particularly at high structure counts. An assumption that the structure counting data are distributed according to the Poisson distribution can therefore lead to confidence intervals narrower than are justified by the data. Moreover, if the Poisson distribution is assumed, the variance is related only to the total number of structures counted. Thus, a particular structure count conducted on one grid opening is considered to have the same confidence interval as that for the same number of structures found on many grid openings. However, the area of sample actually counted is very small in relation to the total area of the filter, and for this reason, structures shall be counted on a minimum of four grid openings taken from different areas of the filter in order to ensure that a representative evaluation of the deposit is made.

At high structure counts, where there are adequate numbers of structures per grid opening to allow a sample estimate of the variance to be made, the distribution can be approximated to a Gaussian, with independent values for the mean and variance. Where the sample estimate of variance exceeds that implicit in the Poissonian assumption, use of Gaussian statistics with the variance defined by the actual data is the most conservative approach to calculation of confidence intervals.

At low structure counts, it is not possible to obtain a reliable sample estimate of the variance, and the distribution also becomes asymmetric but not necessarily Poissonian. For 30 structures and below, the distribution becomes asymmetric enough for the fit to a Gaussian to no longer be a reasonable one, and estimates of sample variance are unreliable. Accordingly, for counts below 31 structures, the assumption of a Poisson distribution shall be made for calculation of the confidence intervals.

F.4.3 Example of calculation of Poissonian 95 % confidence intervals

For total structure counts less than 4, the lower 95 % confidence limit corresponds to less than 1 structure. Therefore, it is not meaningful to quote lower confidence interval points for structure counts of less than 4, and the result shall be recorded as "less than" the corresponding one-sided upper 95 % confidence limit of the Poisson distribution, as follows:

- 0 structure \cong 2,99 times the analytical sensitivity
- 1 structure \cong 4,74 times the analytical sensitivity
- 2 structures \cong 6,30 times the analytical sensitivity
- 3 structures \cong 7,75 times the analytical sensitivity

For total counts exceeding 4, the 95 % confidence interval shall be calculated using the values shown in table F.1. Table F.1 gives the upper and lower limits of the two-sided Poissonian 95 % confidence interval for structure counts up to 470.

F.4.4 Example of calculation of Gaussian 95 % confidence intervals

Calculate the sample estimate of variance s^2 using the following equation:

$$s^2 = \frac{\sum_{i=1}^{i=k} (n_i - np_i)^2}{(k-1)}$$

where

- n_i is the number of structures on the i th grid opening;
- n is the total number of structures found in k grid openings;
- p_i is the fraction of the total area examined represented by the i th grid opening;
- k is the number of grid openings examined.

If the mean value of the structure count is calculated to be n , the upper and lower values of the Gaussian 95 % confidence interval are given respectively by

$$L_u = \frac{n}{k} + \frac{ts}{\sqrt{k}}$$

and

$$L_1 = \frac{n}{k} - \frac{ts}{\sqrt{k}}$$

where

- L_u is the upper 95 % confidence limit;
- L_l is the lower 95 % confidence limit;
- n is the total number of structures in all grid openings examined;
- t is the value of Student's test (probability 0,975) for $(k - 1)$ degrees of freedom;
- s is the standard deviation (square root of sample estimate of variance);
- k is the number of grid openings examined.

F.4.5 Summary of procedure for calculation of results

In summary, structure counting data shall be calculated as follows:

No structures detected

The structure concentration shall be reported as less than the concentration equivalent of the one-sided upper 95 % confidence limit of the Poisson distribution. This is equal to 2,99 times the analytical sensitivity.

From 1 to 3 structures

When 1 to 3 structures are counted, the result shall be reported as less than the corresponding one-sided upper 95 % confidence limit for the Poisson distribution. These are

- 1 structure \cong 4,74 times the analytical sensitivity
- 2 structures \cong 6,30 times the analytical sensitivity
- 3 structures \cong 7,75 times the analytical sensitivity

From 4 to 30 structures

The mean structure concentration and the 95 % confidence intervals shall be reported on the basis of the Poissonian assumption, using the values shown in table F.1.

More than 30 structures

When more 30 structures are counted, both the Gaussian 95 % confidence interval and the Poissonian 95 % confidence interval shall be calculated. The larger of these two intervals shall be used to express the precision of the structure concentration. When the Gaussian 95 % confidence interval is selected for data reporting, the Poissonian 95 % confidence interval shall also be mentioned.

F.5 Calculation of structure length, width, and aspect ratio distributions

The distributions all approximate to logarithmic-normal, and therefore the size range intervals for calculation of the distribution shall be spaced logarithmically. The other characteristics required for the choice of size intervals are that they should allow for a sufficient number of size classes, while still retaining a statistically valid number of structures in each class. Interpretation is also facilitated if each size class repeats at 10 intervals, and if 5 μm is a size class boundary. A ratio from one class to the next of 1,468 satisfies all of these requirements and this value shall be used. The distributions, being approximately logarithmic-normal, when presented graphically, shall be plotted using a logarithmic ordinate scale and a Gaussian abscissa.

F.5.1 Calculation of structure length cumulative number distribution

This distribution allows the fraction of the total number of structures either shorter or longer than a given length to be determined. It is calculated using the following equation:

$$C(P)_k = \frac{\sum_{i=1}^{i=k} n_i}{\sum_{i=1}^P n_i} \times 100$$

where

- $C(P)_k$ is the cumulative number percentage of structures which have lengths less than the upper bound of the k th class;
- n_i is the number of structures in the i th length class;
- P is the total number of length classes.

F.5.2 Calculation of structure width cumulative number distribution

This distribution allows the fraction of the total number of structures either narrower or wider than a given width to be determined. It is calculated in a similar way to that used in F.5.1, but using the structure widths.

F.5.3 Calculation of structure aspect ratio cumulative number distribution

This distribution allows the fraction of the total number of structures which have aspect ratios either smaller or larger than a given aspect ratio to be determined. It is calculated in a similar way to that used in F.5.1, but using the structure aspect ratios.

Table F.1 — Upper and lower limits of the Poissonian 95 % confidence interval of a count

Structure count	Lower limit	Upper limit	Structure count	Lower limit	Upper limit	Structure count	Lower limit	Upper limit
0	0	3,689 ¹⁾	46	33,678	61,358	92	74,164	112,83
1	0,025	5,572	47	34,534	62,501	93	75,061	113,94
2	0,242	7,225	48	35,392	63,642	94	75,959	115,04
3	0,619	8,767	49	36,251	64,781	95	76,858	116,14
4	1,090	10,242	50	37,112	65,919	96	77,757	117,24
5	1,624	11,669	51	37,973	67,056	97	78,657	118,34
6	2,202	13,060	52	38,837	68,192	98	79,557	119,44
7	2,814	14,423	53	39,701	69,326	99	80,458	120,53
8	3,454	15,764	54	40,567	70,459	100	81,360	121,66
9	4,115	17,085	55	41,433	71,591	110	90,400	132,61
10	4,795	18,391	56	42,301	72,721	120	99,490	143,52
11	5,491	19,683	57	43,171	73,851	130	108,61	154,39
12	6,201	20,962	58	44,041	74,979	140	117,77	165,23
13	6,922	22,231	59	44,912	76,106	150	126,96	176,04
14	7,654	23,490	60	45,785	77,232	160	136,17	186,83
15	8,396	24,741	61	46,058	78,357	170	145,41	197,59
16	9,146	25,983	62	47,533	79,482	180	154,66	208,33
17	9,904	27,219	63	48,409	80,605	190	163,94	219,05
18	10,668	28,448	64	49,286	81,727	200	173,24	229,75
19	11,440	29,671	65	50,164	82,848	210	182,56	240,43
20	12,217	30,889	66	51,042	83,969	220	191,89	251,10
21	13,00	32,101	67	51,922	85,088	230	201,24	261,75
22	13,788	33,309	68	52,803	86,207	240	210,60	272,39
23	14,581	34,512	69	53,685	87,324	250	219,97	283,01
24	15,378	35,711	70	54,567	88,441	260	229,36	293,62
25	16,178	36,905	71	55,451	89,557	270	238,75	304,23
26	16,983	38,097	72	56,335	90,673	280	248,16	314,82
27	17,793	39,284	73	57,220	91,787	290	257,58	325,39
28	18,606	40,468	74	58,106	92,901	300	267,01	335,96
29	19,422	41,649	75	58,993	94,014	310	276,45	346,52
30	20,241	42,827	76	59,880	95,126	320	285,90	357,08
31	21,063	44,002	77	60,768	96,237	330	295,36	367,62
32	21,888	45,175	78	61,657	97,348	340	304,82	378,15
33	22,715	46,345	79	62,547	98,458	350	314,29	388,68
34	23,545	47,512	80	63,437	99,567	360	323,77	399,20
35	24,378	48,677	81	64,328	100,68	370	333,26	409,71
36	25,213	49,840	82	65,219	101,79	380	342,75	420,22
37	26,050	51,000	83	66,111	102,90	390	352,25	430,72
38	26,890	52,158	84	67,003	104,00	400	361,76	441,21
39	27,732	53,315	85	67,897	105,11	410	371,27	451,69
40	28,575	54,469	86	68,790	106,21	420	380,79	462,18
41	29,421	55,622	87	69,684	107,32	430	390,32	472,65
42	30,269	56,772	88	70,579	108,42	440	399,85	483,12
43	31,119	57,921	89	71,474	109,53	450	409,38	493,58
44	31,970	59,068	90	72,370	110,63	460	418,92	504,04
45	32,823	60,214	91	73,267	111,73	470	428,47	514,50

1) The one-sided upper 95 % confidence limit for 0 structures is 2,99.

Annex G (informative)

Strategies for collection of air samples

G.1 General

An important part of the sampling strategy is a statement of the purpose of the sampling programme. A sufficient number of samples should be collected so that the site is well characterized to the precision and accuracy desired, and also ensure that sample filters appropriately loaded for TEM analysis are obtained from all of the sampling locations.

G.2 Air sample collection in the outdoors environment

Weather conditions restrict the ability to collect satisfactory air samples in the outdoors environment, and whenever possible, sampling should be carried out in low-wind, low-humidity conditions. Detailed records of the weather conditions, windspeed and direction during the sampling period should be made. All available information concerning local topography, and the types and positions of sources should be recorded.

Sequential multipoint sampling is necessary to provide adequate characterization of complex sites and sources. It is recommended that multiple samples are taken upwind and downwind of the site, with a mini-

mum of two samples in the downwind position expected to experience the maximum airborne concentration. The locations of the samplers should be carefully recorded.

G.3 Air sample collection inside buildings

Air samples are often collected inside buildings in which asbestos-containing construction materials are present, in order to determine whether these materials contribute to the asbestos fibre concentration in the building atmosphere. The optimum positions for collection of air samples can only be determined after a complete survey of the building to establish air movement patterns. Multiple samples should be collected in the area where asbestos building materials are present, and control samples should be collected in an adjacent area where no airborne asbestos fibres would be expected. The intakes for air conditioning systems are frequently used as the collection locations for control samples. Whenever possible, static samples should be taken over a period exceeding 4 h during normal activity in the building, at face velocities of between 4 cm/s and 25 cm/s.

Annex H

(informative)

Methods for removal of gypsum fibres

It is common to find fibres of calcium sulfate (gypsum) in airborne particulates collected in buildings and urban environments, and particularly in samples collected where demolition or construction work is in progress. The fibres are readily released when plasters and cement products are disturbed. In some circumstances, particles of calcite or dolomite collected on an air filter can react with atmospheric sulfur dioxide, to form long fibres of gypsum. Gypsum fibres can give rise to high fibre counts by both optical and electron microscopy. The gypsum fibres are often 2 µm to 6 µm long, with aspect ratios greater than 10/1. Sometimes, these fibres appear similar to amphibole asbestos fibres, and in some samples they can be morphologically very similar to chrysotile. In the TEM, the larger fibres have high contrast and at high magnification often exhibit a characteristic mottled appearance which changes under electron beam irradiation. Some gypsum fibres, however, are not easily discriminated from asbestos without examination by EDXA. TEM specimens which contain many such gypsum fibres require an extended examination time in the TEM, because it is necessary to

examine each of these fibres by EDXA before it can be rejected.

It is possible to remove gypsum fibres selectively by water extraction. A Jaffe washer (7.3.7), or a condensation washer (7.3.8), should be prepared, but using a water (6.1) as the solvent. The TEM specimens, which have been previously prepared and initially examined in the TEM, should be placed in the washer to allow dissolution of the fibres. If a Jaffe washer is used, the treatment time can be reduced by heating the washer to 90 °C to 100 °C for a few minutes. If a condensation washer is used, the gypsum fibres will be dissolved by treatment for approximately 10 min. The effect of this treatment is to remove the gypsum fibres, leaving carbon replicas (7.3.11) which are readily distinguished from asbestos fibres.

NOTE 20 This procedure should be used only when examination of the untreated TEM specimen grids shows the gypsum fibres to be isolated from any asbestos fibres present. Losses of asbestos fibres may occur if matrices of gypsum and asbestos are exposed to this procedure.

Annex J (informative)

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